SOCIETY FOR NEUROSCIENCE

ABSTRACTS

VOLUME 9, PART 2

13th ANNUAL MEETING
BOSTON, MASSACHUSETTS
NOVEMBER 6–11, 1983

Made in the United States of America
International Standard Book Numbers
Part 1 0-916110-13-3
Part 2 0-916110-14-1
Both Parts 0-916110-15-X
Library of Congress Catalog Card Number 75-7761

Proper citation form for this volume:
Soc. Neurosci. Abstr., Vol. 9, Part 2, p. xxx, 1983.

Published by
Society for Neuroscience
9650 Rockville Pike
Bethesda, Maryland 20814

CONTENTS—PART 2

	Page
Program Committee	**iv**
Chronological List of Sessions	**v**
Thematic List of Sessions	**vii**
Abstracts in Session Order*	
Wednesday, November 9–Friday, November 11	**759-1248**
Author Index	**1249**
Key Word Index	**1273**

***4764 volunteer abstracts, 20 symposium and workshop abstracts.**

1983 PROGRAM COMMITTEE

John G. Hildebrand, *Chair*
Columbia University

Neal H. Barmack
Neurological Sciences Institute

Allan I. Basbaum
University of California

Michael V. L. Bennett
Albert Einstein College of Medicine

Ira B. Black
Cornell University

Marc Colonnier
Hospital of L'Enfant-Jesus

Martha Constantine-Paton
Princeton University

Joseph T. Coyle
Johns Hopkins University School of Medicine

Irving Kupfermann
Columbia University

Dennis M. D. Landis
Massachusetts General Hospital

Eduardo R. Macagno
Columbia University

Peter R. MacLeish
Harvard Medical School

Mortimer Mishkin
Laboratory of Neuropsychology, NIMH

Paul H. O'Lague
University of California

Edward M. Schmidt
Laboratory of Neural Control, NIH-NINCDS

Lawrence P. Schramm
Johns Hopkins University School of Medicine

James H. Schwartz
Columbia University College of Physicians and Surgeons

Michael J. Brownstein, *Consultant*
Laboratory of Clinical Science, NIH

David H. Cohen, *ex officio*
State University of New York

Jennifer H. LaVail, *ex officio*
University of California School of Medicine

Dominick P. Purpura, *ex officio*
Stanford University School of Medicine

CHRONOLOGICAL LIST OF SESSIONS

CONTINUED FROM SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 9, PART 1

(see page vii for Thematic List of Sessions.)

Session Number and Title	Page

WEDNESDAY

Slide Sessions—1:00 PM

223. Development and plasticity: retinotectal system II .. 759
224. Epilepsy: kindling II 762
225. Subcortical auditory pathways III 765
226. Regeneration: central III 768
227. Cardiovascular regulation: central transmitters II ... 772

Poster Sessions—1:00 PM

228. Skin, muscle, joint, and visceral receptors 775
229. Pain: afferent nociceptors 779
230. Pain: central pathways 782
231. Pain modulation: anatomy and pharmacology 785
232. Pain modulation: pharmacology 790
233. Opiates, endorphins, and enkephalins: physiological effects III 796
234. Retina II 800
235. Retina and retinal projections 809
236. Subcortical visual pathways I 813
237. Subcortical visual pathways II 817
238. Visual cortex: intrinsic organization III 820
239. Learning and memory: pharmacology II 824
240. Learning and memory: pharmacology of conditioning 828
241. Neurogenetics 832
242. Development of invertebrates II 834
243. Development and plasticity: trophic agents II 836
244. Development and plasticity: trophic interactions II 842
245. Morphogenesis and pattern formation 847
246. Cell lineage and differentiation I 852
247. Synapse elimination, competition, and neuron death II 855
248. Neural plasticity in adult animals III 859
249. Muscle afferents 862
250. Vestibular, visual, and oculomotor plasticity 865
251. Cerebellum: anatomical studies 869
252. Basal ganglia: behavior and pharmacology II 873
253. Regional neuropharmacology 877
254. Presynaptic mechanisms I 879
255. Blood-brain barrier II 885

Special Lectures—5:15 PM

256. The regulation of neuroendocrine gene expression. J. P. ROSENFELD ..... No abstract
257. The memory trace found? Neural substrates of basic associative learning in the mammalian brain. R. F. THOMPSON ..... No abstract
258. The ontogeny of the peripheral nervous system studied with an avian model. N. LEDOUARIN ..... No abstract

Session Number and Title	Page

THURSDAY

Symposia—8:30 AM

259. Post-translational processing of neuropeptide precursors and neuronal proteins. *Chaired:* H. GAINER 890
260. Efferent control of the organs of hearing and equilibrium. *Chaired:* S. M. HIGHSTEIN 890

Slide Sessions—8:30 AM

261. Opiates III 890
262. Retina III 894
263. Cell lineage and differentiation II 897
264. Feeding and drinking: cues for need state III 900
265. Peripheral autonomic nervous system II 903
266. Epilepsy: physiology II 906
267. Visual cortical development 910
268. Invertebrate learning and behavior II 913
269. Human neuropsychology II 916
270. Somatosensory system 920

Poster Sessions—8:30 AM

271. Aging I 923
272. Aging II 928
273. Development and plasticity: denervation 932
274. Development and plasticity: autonomic nervous system 935
275. Developmental disorders 938
276. Development and plasticity: biochemical and pharmacological correlates I 942
277. Development and plasticity: limbic system 946
278. Basal ganglia: physiology 949
279. Auditory cortex 953
280. Visual cortex: extrastriate visual areas II 957
281. Acetylcholine: biosynthesis and regulation 959
282. Acetylcholine: cellular structures and projection pathways 963
283. Acetylcholine: biosynthesis and electrophysiology .. 967
284. Brain metabolism I 971
285. Motivation and emotion: self-stimulation 976
286. Motivation and emotion 978
287. Sprouting I 982
288. Sprouting II 985
289. Catecholamines: biochemical characterization I 989
290. Catecholamines: biochemical characterization II 994
291. Catecholamines: adrenergic physiology 999
292. Catecholamines: dopaminergic physiology 1005
293. Opiates, endorphins, and enkephalins: receptors II 1010
294. Peptides: anatomical localization III 1014
295. Chemical senses: olfaction and taste II 1019
296. Synaptic structure and function I 1025

Session Number and Title	Page
297. Control of posture and movement II	1031
298. Disorders of the motor system: neural prostheses	1035
Symposia—1:00 PM	
299. Clocks in the test tube: toward a mechanistic analysis of circadian oscillators. *Chaired:* M. MENAKER	1039
300. Biochemistry of synaptic regulation. *Chaired:* M. B. KENNEDY and R. B. KELLY	1039
Slide Sessions—1:00 PM	
301. GABA and benzodiazepines: binding sites II	1039
302. Process outgrowth, growth cones, and guidance mechanisms II	1043
303. Subcortical visual pathways III	1046
304. Presynaptic mechanisms II	1049
305. Transmitters and receptors in disease II	1052
306. Colocalization of transmitters	1055
307. Stress, hormones, and the autonomic nervous system II	1057
308. Developmental specificity	1058
309. Aging III	1060
310. Pain modulation: behavioral analysis	1061
Poster Sessions—1:00 PM	
311. Comparative neuroanatomy I	1063
312. Biological rhythms: suprachiasmatic nucleus	1067
313. Biological rhythms II	1072
314. Hormonal control of behavior I	1076
315. Hormonal control of behavior II	1079
316. Sensorimotor integration	1082
317. Oculomotor brainstem mechanisms	1086
318. Cerebellum: transmitters and histochemistry	1090
319. Development and plasticity: endocrine control	1093
320. Development and plasticity: biochemical and pharmacological correlates II	1097
321. Diseases of synapses and axons	1102
322. Epilepsy: miscellany	1104
323. Catecholamines: dopamine receptors II	1108
324. Catecholamines: dopamine receptors III	1112
325. Catecholamines: adrenergic receptors	1117
326. Stress, hormones, and the autonomic nervous system III	1122
327. Modulators and modulations	1125
328. Opiates, endorphins, and enkephalins: physiological effects IV	1128
329. Peptides: biosynthesis and metabolism II	1132
330. Effects of drugs on receptors	1137
331. Drugs of abuse: nonopiates II	1145
332. Catecholamines: anatomical localization	1149
333. Biogenic amines II	1152
334. Cardiovascular regulation: morphological aspects	1157
335. Respiratory regulation	1160

Session Number and Title	Page
FRIDAY	
Symposia—8:30 AM	
336. The oculomotor role of the accessory optic system and pretectum: Is there a single vertebrate scheme? *Chaired:* J. WALLMAN	1165
337. Corticotropin releasing factor. *Chaired:* W. W. VALE	No abstract
Slide Sessions—8:30 AM	
338. Comparative neuroanatomy II	1165
339. Brain metabolism II	1168
340. Monoamines and behavior III	1172
341. Synaptic structure and function II	1175
342. Synaptogenesis II	1178
343. Immunohistochemistry of specific cellular components	1181
344. Excitatory amino acids II	1184
345. Membrane biophysics II	1187
346. Axoplasmic transport III	1190
Poster Sessions—8:30 AM	
347. Evoked potentials and EEG	1193
348. Sleep	1201
349. Peptides: receptors III	1205
350. Neuromodulators	1208
351. Subcortical organization	1213
352. Development of the visual system: cortex	1216
353. Visual cortex: cortico-cortical relationships	1219
354. Neural plasticity in adult animals IV	1221
355. Muscle II	1225
356. Basal ganglia: thalamic relationships	1229
357. Alcohol: environmental influences and electrophysiology	1231
358. Alcohol and barbiturates: biochemistry	1233
359. Alcohol: behavior	1239
360. Neurotoxicity III	1244

Sessions end at 12:45 PM, Friday, November 11.

THEMATIC LIST OF SESSIONS

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

Theme A: Development and Plasticity

Session Number	Session Title	Type	Day	Time
271.	Aging I	Poster	Thu	8.30 AM
272.	Aging II	Poster	Thu	8:30 AM
309.	Aging III	Slide	Thu	1:00 PM
246.	Cell lineage and differentiation I	Poster	Wed	1:00 PM
263.	Cell lineage and differentiation II	Slide	Thu	8:30 AM
44.	Cell recognition in development of the nervous system	**Symp.**	Mon	1:00 PM
274.	Development and plasticity: autonomic nervous system	Poster	Thu	8:30 AM
276.	Development and plasticity: biochemical and pharmacological correlates I	Poster	Thu	8:30 AM
320.	Development and plasticity: biochemical and pharmacological correlates II	Poster	Thu	1:00 PM
273.	Development and plasticity: denervation	Poster	Thu	8:30 AM
319.	Development and plasticity: endocrine control	Poster	Thu	1:00 PM
277.	Development and plasticity: limbic system	Poster	Thu	8:30 AM
17.	Development and plasticity: peripheral regeneration	Poster	Mon	8:30 AM
19.	Development and plasticity: retinotectal system I	Poster	Mon	8:30 AM
223.	Development and plasticity: retinotectal system II	Slide	Wed	1:00 PM
112.	Development and plasticity: sensory systems	Poster	Tue	8:30 AM
182.	Development and plasticity: trophic agents I	Slide	Wed	8:30 AM
243.	Development and plasticity: trophic agents II	Poster	Wed	1:00 PM
6.	Development and plasticity: trophic interactions I	Slide	Mon	8:30 AM
244.	Development and plasticity: trophic interactions II	Poster	Wed	1:00 PM
179.	Development of invertebrates I	Slide	Wed	8:30 AM
242.	Development of invertebrates II	Poster	Wed	1:00 PM
20.	Development of motor systems	Poster	Mon	8:30 AM
352.	Development of the visual system: cortex	Poster	Fri	8:30 AM
206.	Development of the visual system: retina and geniculate	Poster	Wed	8:30 AM
11.	Development of the visual system: retinothalamic projections	Slide	Mon	8:30 AM
92.	Development: endocrine control and transmitter plasticity	Slide	Tue	8:30 AM
275.	Developmental disorders	Poster	Thu	8:30 AM
308.	Developmental specificity	Slide	Thu	1:00 PM
132.	Developmental strategies for selective synapse formation	**Symp.**	Tue	1:00 PM
18.	Effects of activity and inactivity on development	Poster	Mon	8:30 AM
245.	Morphogenesis and pattern formation	Poster	Wed	1:00 PM
30.	Neural plasticity in adult animals I	Poster	Mon	8:30 AM
113.	Neural plasticity in adult animals II	Poster	Tue	8:30 AM
248.	Neural plasticity in adult animals III	Poster	Wed	1:00 PM
354.	Neural plasticity in adult animals IV	Poster	Fri	8:30 AM
241.	Neurogenetics	Poster	Wed	1:00 PM
78.	Neurotoxicity I	Poster	Mon	1:00 PM
198.	Neurotoxicity II	Poster	Wed	8:30 AM
360.	Neurotoxicity III	Poster	Fri	8:30 AM
151.	Nutritional and prenatal factors in development I	Poster	Tue	1:00 PM
197.	Nutritional and prenatal factors in development II	Poster	Wed	8:30 AM
64.	Process outgrowth, growth cones, and guidance mechanisms I	Poster	Mon	1:00 PM

302.	Process outgrowth, growth cones, and guidance mechanisms II	Slide	Thu	1:00 PM
204.	Regeneration: central I	Poster	Wed	8:30 AM
205.	Regeneration: central II	Poster	Wed	8:30 AM
226.	Regeneration: central III	Slide	Wed	1:00 PM
111.	Specificity of synaptic connections	Poster	Tue	8:30 AM
287.	Sprouting I	Poster	Thu	8:30 AM
288.	Sprouting II	Poster	Thu	8:30 AM
97.	Synapse elimination, competition, and neuron death I	Slide	Tue	8:30 AM
247.	Synapse elimination, competition, and neuron death II	Poster	Wed	1:00 PM
203.	Synaptogenesis I	Poster	Wed	8:30 AM
342.	Synaptogenesis II	Slide	Fri	8:30 AM
218.	The neurogenetics of identified cells	**Symp.**	Wed	1:00 PM
267.	Visual cortical development	Slide	Thu	8:30 AM

Theme B: Cell Biology

Session Number	Session Title	Type	Day	Time
42.	Axoplasmic transport I	Poster	Mon	8:30 AM
43.	Axoplasmic transport II	Poster	Mon	8:30 AM
346.	Axoplasmic transport III	Slide	Fri	8:30 AM
49.	Blood-brain barrier I	Slide	Mon	1:00 PM
255.	Blood-brain barrier II	Poster	Wed	1:00 PM
222.	Cell surface and related components	Slide	Wed	1:00 PM
103.	Cell surface, cytoskeleton, and related macromolecules	Poster	Tue	8:30 AM
72.	Cell and tissue culture: behavior of neural, glia, muscle, and model cells	Poster	Mon	1:00 PM
5.	Cell and tissue culture: neurons, glia, and neuronal models	Slide	Mon	8:30 AM
172.	Cellular aspects of disease	Poster	Tue	1:00 PM
104.	Cellular localization of receptors	Poster	Tue	8:30 AM
131.	Functions of glia	Poster	Tue	8:30 AM
88.	Identified cells	Poster	Mon	1:00 PM
343.	Immunohistochemistry of specific cellular components	Slide	Fri	8:30 AM
71.	Lipids, myelin, and glial proteins	Poster	Mon	1:00 PM
105.	Membrane structure and function	Poster	Tue	8:30 AM
173.	Metabolic studies	Poster	Tue	1:00 PM
184.	Molecular biology of gene expression	Slide	Wed	8:30 AM
102.	Morphology of neurons and glia	Poster	Tue	8:30 AM
91.	New approaches to the study of the mechanism of fast axonal transport	**Wksh.**	Tue	8:30 AM
87.	Staining and tracing techniques	Poster	Mon	1:00 PM
27.	Structure and function of the neuroendocrine cell	Poster	Mon	8:30 AM
176.	The acidic interior of secretory vesicles: mechanisms and implications for neurobiology	**Symp.**	Wed	8:30 AM

Theme C: Excitable Membranes and Synaptic Transmission

Session Number	Session Title	Type	Day	Time
10.	Action potential and ion channels I	Slide	Mon	8:30 AM
147.	Action potential and ion channels II	Poster	Tue	1:00 PM
148.	Action potential and ion channels III	Poster	Tue	1:00 PM
199.	Action potential and ion channels IV	Poster	Wed	8:30 AM

300.	Biochemistry of synaptic regulation	**Symp.**	Thu	1:00 PM
321.	Diseases of synapses and axons	Poster	Thu	1:00 PM
330.	Effects of drugs on receptors	Poster	Thu	1:00 PM
178.	Electrophysiology of CNS neuron	Slide	Wed	8:30 AM
200.	Electrophysiology of CNS neurons II	Poster	Wed	8:30 AM
322.	Epilepsy: miscellany	Poster	Thu	1:00 PM
118.	Epilepsy: physiology I	Poster	Tue	8:30 AM
266.	Epilepsy: physiology II	Slide	Thu	8:30 AM
23.	Excitable membranes and synaptic transmission: invertebrate studies	Poster	Mon	8:30 AM
149.	Membrane biophysics I	Poster	Tue	1:00 PM
345.	Membrane biophysics II	Slide	Fri	8:30 AM
70.	Membrane biophysics: voltage clamp and model analysis	Poster	Mon	1:00 PM
69.	Pharmacology of synaptic transmission	Poster	Mon	1:00 PM
135.	Postsynaptic mechanisms I	Slide	Tue	1:00 PM
213.	Postsynaptic mechanisms II	Poster	Wed	8:30 AM
254.	Presynaptic mechanisms I	Poster	Wed	1:00 PM
304.	Presynaptic mechanisms II	Slide	Thu	1:00 PM
50.	Sensory transduction I	Slide	Mon	1:00 PM
296.	Synaptic structure and function I	Poster	Thu	8:30 AM
341.	Synaptic structure and function II	Slide	Fri	8:30 AM

Theme D: Neurotransmitters, Modulators, and Receptors

Session Number	Session Title	Type	Day	Time
283.	Acetylcholine: biosynthesis and electrophysiology	Poster	Thu	8:30 AM
281.	Acetylcholine: biosynthesis and regulation	Poster	Thu	8:30 AM
282.	Acetylcholine: cellular structures and projection pathways	Poster	Thu	8:30 AM
38.	Behavioral pharmacology I	Poster	Mon	8:30 AM
39.	Behavioral pharmacology II	Poster	Mon	8:30 AM
36.	Behavioral pharmacology: dopamine	Poster	Mon	8:30 AM
37.	Behavioral pharmacology: serotonin	Poster	Mon	8:30 AM
101.	Biogenic amines I	Slide	Tue	8:30 AM
333.	Biogenic amines II	Poster	Thu	1:00 PM
291.	Catecholamines: adrenergic physiology	Poster	Thu	8:30 AM
325.	Catecholamines: adrenergic receptors	Poster	Thu	1:00 PM
332.	Catecholamines: anatomical localization	Poster	Thu	1:00 PM
289.	Catecholamines: biochemical characterization I	Poster	Thu	8:30 AM
290.	Catecholamines: biochemical characterization II	Poster	Thu	8:30 AM
13.	Catecholamines: dopamine receptors I	Slide	Mon	8:30 AM
323.	Catecholamines: dopamine receptors II	Poster	Thu	1:00 PM
324.	Catecholamines: dopamine receptors III	Poster	Thu	1:00 PM
292.	Catecholamines: dopaminergic physiology	Poster	Thu	8:30 AM
48.	Characterization of cholinergic receptors	Slide	Mon	1:00 PM
169.	Characterization of muscarinic receptors	Poster	Tue	1:00 PM
168.	Characterization of nicotinic receptors	Poster	Tue	1:00 PM
166.	Characterization of noncholinergic receptors	Poster	Tue	1:00 PM
167.	Coexistence of transmitters and neuromodulators	Poster	Tue	1:00 PM
306.	Colocalization of transmitters	Slide	Thu	1:00 PM
26.	Cyclic nucleotides	Poster	Mon	8:30 AM
216.	Excitatory amino acid neurotransmitters	**Symp.**	Wed	1:00 PM
77.	Excitatory amino acids I	Poster	Mon	1:00 PM
344.	Excitatory amino acids II	Slide	Fri	8:30 AM
120.	GABA and benzodiazepines: binding sites I	Poster	Tue	8:30 AM

301.	GABA and benzodiazepines: binding sites II	Slide	Thu	1:00 PM
121.	GABA and benzodiazepines: CNS distribution of binding sites	Poster	Tue	8:30 AM
122.	GABA and benzodiazepines: electrophysiology and behavior	Poster	Tue	8:30 AM
133.	Heterogeneity of neurotransmitter receptors	**Wksh.**	Tue	1:00 PM
209.	Interaction between neurotransmitters	Poster	Wed	8:30 AM
86.	Metabolism of transmitters and modulators	Poster	Mon	1:00 PM
327.	Modulators and modulations	Poster	Thu	1:00 PM
45.	Monoaminergic innervation of cortex: new evidence of anatomical and physiological specificity	**Symp.**	Mon	1:00 PM
350.	Neuromodulators	Poster	Fri	8:30 AM
129.	Opiates, endorphins, and enkephalins: anatomical localization	Poster	Tue	8:30 AM
170.	Opiates, endorphins, and enkephalins: biochemical characterization	Poster	Tue	1:00 PM
84.	Opiates, endorphins, and enkephalins: physiological effects I	Poster	Mon	1:00 PM
215.	Opiates, endorphins, and enkephalins: physiological effects II	Poster	Wed	8:30 AM
233.	Opiates, endorphins, and enkephalins: physiological effects III	Poster	Wed	1:00 PM
328.	Opiates, endorphins, and enkephalins: physiological effects IV	Poster	Thu	1:00 PM
99.	Opiates, endorphins, and enkephalins: receptors I	Slide	Tue	8:30 AM
293.	Opiates, endorphins, and enkephalins: receptors II	Poster	Thu	8:30 AM
94.	Peptide transmitters in invertebrates	Slide	Tue	8:30 AM
85.	Peptides: anatomical localization I	Poster	Mon	1:00 PM
134.	Peptides: anatomical localization II	Slide	Tue	1:00 PM
294.	Peptides: anatomical localization III	Poster	Thu	8:30 AM
40.	Peptides: biochemical characterization	Poster	Mon	8:30 AM
219.	Peptides: biosynthesis and metabolism I	Slide	Wed	1:00 PM
329.	Peptides: biosynthesis and metabolism II	Poster	Thu	1:00 PM
9.	Peptides: physiological effects I	Slide	Mon	8:30 AM
41.	Peptides: physiological effects II	Poster	Mon	8:30 AM
130.	Peptides: physiological effects III	Poster	Tue	8:30 AM
52.	Peptides: receptors I	Slide	Mon	1:00 PM
171.	Peptides: receptors II	Poster	Tue	1:00 PM
349.	Peptides: receptors III	Poster	Fri	8:30 AM
259.	Post-translational processing of neuropeptide precursors and neuronal proteins	**Symp.**	Thu	8:30 AM
53.	Regional localization of receptors and transmitters I	Slide	Mon	1:00 PM
211.	Regional localization of receptors and transmitters II	Poster	Wed	8:30 AM
175.	The dynorphin peptides	**Symp.**	Wed	8:30 AM
25.	Transmitter immunocytochemistry	Poster	Mon	8:30 AM
164.	Transmitter uptake, storage, and secretion I	Poster	Tue	1:00 PM
165.	Transmitter uptake, storage, and secretion II	Poster	Tue	1:00 PM
210.	Transmitters and receptors in disease I	Poster	Wed	8:30 AM
305.	Transmitters and receptors in disease II	Slide	Thu	1:00 PM
24.	Transmitters in invertebrates	Poster	Mon	8:30 AM

Theme E: Endocrine and Autonomic Regulation

Session Number	Session Title	Type	Day	Time
116.	Adrenal medulla	Poster	Tue	8:30 AM
160.	Cardiovascular regulation: central transmitters I	Poster	Tue	1:00 PM

227.	Cardiovascular regulation: central transmitters II	Slide	Wed	1:00 PM
31.	Cardiovascular regulation: functional aspects I	Poster	Mon	8:30 AM
55.	Cardiovascular regulation: functional aspects II	Slide	Mon	1:00 PM
159.	Cardiovascular regulation: hypertension and stress	Poster	Tue	1:00 PM
334.	Cardiovascular regulation: morphological aspects	Poster	Thu	1:00 PM
337.	Corticotropin releasing factor	**Symp.**	Fri	8:30 AM
96.	Endocrine control: central pathways I	Slide	Tue	8:30 AM
123.	Endocrine control: central pathways II	Poster	Tue	8:30 AM
35.	Endocrine and autonomic regulation: brain ventricular system	Poster	Mon	8:30 AM
34.	Endocrine and autonomic regulation: neural control of immune system	Poster	Mon	8:30 AM
314.	Hormonal control of behavior I	Poster	Thu	1:00 PM
315.	Hormonal control of behavior II	Poster	Thu	1:00 PM
32.	Peripheral autonomic nervous system I	Poster	Mon	8:30 AM
265.	Peripheral autonomic nervous system II	Slide	Thu	8:30 AM
212.	Pineal gland	Poster	Wed	8:30 AM
33.	Regulation of autonomic function I	Poster	Mon	8:30 AM
181.	Regulation of autonomic function II	Slide	Wed	8:30 AM
117.	Regulation of pituitary function I	Poster	Tue	8:30 AM
136.	Regulation of pituitary function II	Slide	Tue	1:00 PM
207.	Regulation of pituitary function III	Poster	Wed	8:30 AM
208.	Regulation of pituitary function IV	Poster	Wed	8:30 AM
335.	Respiratory regulation	Poster	Thu	1:00 PM

Theme F: Sensory Systems

Session Number	Session Title	Type	Day	Time
279.	Auditory cortex	Poster	Thu	8:30 AM
137.	Chemical senses: olfaction and taste I	Slide	Tue	1:00 PM
295.	Chemical senses: olfaction and taste II	Poster	Thu	8:30 AM
16.	Cochlea	Poster	Mon	8:30 AM
260.	Efferent control of the organs of hearing and equilibrium	**Symp.**	Thu	8:30 AM
109.	Evoked potentials	Poster	Tue	8:30 AM
110.	Evoked potentials: visual	Poster	Tue	8:30 AM
90.	Lesions of the visual system during infancy or adulthood: effects on morphology, physiology, and behavior	**Symp.**	Tue	8:30 AM
231.	Pain modulation: anatomy and pharmacology	Poster	Wed	1:00 PM
310.	Pain modulation: behavioral analysis	Slide	Thu	1:00 PM
4.	Pain modulation: bulbospinal mechanisms	Slide	Mon	8:30 AM
232.	Pain modulation: pharmacology	Poster	Wed	1:00 PM
229.	Pain: afferent nociceptors	Poster	Wed	1:00 PM
230.	Pain: central pathways	Poster	Wed	1:00 PM
202.	Retina I	Poster	Wed	8:30 AM
234.	Retina II	Poster	Wed	1:00 PM
262.	Retina III	Slide	Thu	8:30 AM
235.	Retina and retinal projections	Poster	Wed	1:00 PM
66.	Sensory systems in invertebrates I	Poster	Mon	1:00 PM
98.	Sensory systems in invertebrates II	Slide	Tue	8:30 AM
201.	Sensory transduction II	Poster	Wed	8:30 AM
228.	Skin, muscle, joint, and visceral receptors	Poster	Wed	1:00 PM
74.	Somatosensory cortex and thalamo-cortical relationships	Poster	Mon	1:00 PM
270.	Somatosensory system	Slide	Thu	8:30 AM

76.	Spinal cord: afferent input and local circuits	Poster	Mon	1:00 PM
75.	Spinal cord: cytochemistry and pharmacology	Poster	Mon	1:00 PM
140.	Spinal cord: somatosensory physiology and behavior	Slide	Tue	1:00 PM
65.	Subcortical auditory pathways I	Poster	Mon	1:00 PM
146.	Subcortical auditory pathways II	Poster	Tue	1:00 PM
225.	Subcortical auditory pathways III	Slide	Wed	1:00 PM
73.	Subcortical somatosensory pathways	Poster	Mon	1:00 PM
236.	Subcortical visual pathways I	Poster	Wed	1:00 PM
237.	Subcortical visual pathways II	Poster	Wed	1:00 PM
303.	Subcortical visual pathways III	Slide	Thu	1:00 PM
336.	The oculomotor role of the accessory optic system and pretectum: Is there a single vertebrate scheme?	**Symp.**	Fri	8:30 AM
214.	Vestibular sensory organs	Poster	Wed	8:30 AM
353.	Visual cortex: cortico-cortical relationships	Poster	Fri	8:30 AM
14.	Visual cortex: cortico-subcortical relationships	Poster	Mon	8:30 AM
46.	Visual cortex: extrastriate visual areas I	Slide	Mon	1:00 PM
280.	Visual cortex: extrastriate visual areas II	Poster	Thu	8:30 AM
141.	Visual cortex: intrinsic organization I	Slide	Tue	1:00 PM
183.	Visual cortex: intrinsic organization II	Slide	Wed	8:30 AM
238.	Visual cortex: intrinsic organization III	Poster	Wed	1:00 PM

Theme G: Systems and Sensorimotor Integration

Session Number	Session Title	Type	Day	Time
217.	Applications of neuroscience to human prostheses	**Symp.**	Wed	1:00 PM
8.	Basal ganglia: anatomy and physiology I	Slide	Mon	8:30 AM
195.	Basal ganglia: anatomy and physiology II	Poster	Wed	8:30 AM
143.	Basal ganglia: behavior and pharmacology I	Slide	Tue	1:00 PM
252.	Basal ganglia: behavior and pharmacology II	Poster	Wed	1:00 PM
278.	Basal ganglia: physiology	Poster	Thu	8:30 AM
356.	Basal ganglia: thalamic relationships	Poster	Fri	8:30 AM
251.	Cerebellum: anatomical studies	Poster	Wed	1:00 PM
68.	Cerebellum: microelectrode recordings	Poster	Mon	1:00 PM
180.	Cerebellum: olivocerebellar function	Slide	Wed	8:30 AM
318.	Cerebellum: transmitters and histochemistry	Poster	Thu	1:00 PM
187.	Control of limb movements	Poster	Wed	8:30 AM
21.	Control of posture	Poster	Mon	8:30 AM
54.	Control of posture and movement I	Slide	Mon	1:00 PM
297.	Control of posture and movement II	Poster	Thu	8:30 AM
93.	Cortex I	Slide	Tue	8:30 AM
145.	Cortex II	Poster	Tue	1:00 PM
298.	Disorders of the motor system: neural prostheses	Poster	Thu	8:30 AM
114.	Invertebrate motor function	Poster	Tue	8:30 AM
107.	Locomotion I	Poster	Tue	8:30 AM
188.	Locomotion II	Poster	Wed	8:30 AM
249.	Muscle afferents	Poster	Wed	1:00 PM
7.	Muscle I	Slide	Mon	8:30 AM
355.	Muscle II	Poster	Fri	8:30 AM
317.	Oculomotor brainstem mechanisms	Poster	Thu	1:00 PM
220.	Oculomotor system: cortico-collicular organization	Slide	Wed	1:00 PM
22.	Oculomotor system: mechanics and psychophysics	Poster	Mon	8:30 AM
3.	Reciprocal inhibition and coactivation of antagonist muscles: two fundamental modes of motor control	**Wksh.**	Mon	8:30 AM

139.	Reflex function I	Slide	Tue	1:00 PM
153.	Reflex function II	Poster	Tue	1:00 PM
316.	Sensorimotor integration	Poster	Thu	1:00 PM
83.	Spinal cord and brain stem I	Poster	Mon	1:00 PM
108.	Spinal cord and brain stem II	Poster	Tue	8:30 AM
196.	Spinal cord and brain stem III	Poster	Wed	8:30 AM
152.	Vestibular compensation and brainstem pathways	Poster	Tue	1:00 PM
95.	Vestibular reflexes: functional organization	Slide	Tue	8:30 AM
250.	Vestibular, visual, and oculomotor plasticity	Poster	Wed	1:00 PM

Theme H: Structure and Function of the CNS

Session Number	Session Title	Type	Day	Time
284.	Brain metbolism I	Poster	Thu	8:30 AM
339.	Brain metabolism II	Slide	Fri	8:30 AM
311.	Comparative neuroanatomy I	Poster	Thu	1:00 PM
338.	Comparative neuroanatomy II	Slide	Fri	8:30 AM
15.	Cortex and cortico-subcortical relationships I	Poster	Mon	8:30 AM
106.	Cortex and cortico-subcortical relationships II	Poster	Tue	8:30 AM
79.	Diseases of the CNS	Poster	Mon	1:00 PM
144.	Epilepsy: kindling I	Poster	Tue	1:00 PM
224.	Epilepsy: kindling II	Slide	Wed	1:00 PM
119.	Epilepsy: mutants and toxins	Poster	Tue	8:30 AM
186.	Epilepsy: pharmacology	Slide	Wed	8:30 AM
347.	Evoked potentials and EEG	Poster	Fri	8:30 AM
150.	Limbic system and hypothalamus	Poster	Tue	1:00 PM
67.	Limbic system: hippocampus and amygdala	Poster	Mon	1:00 PM
253.	Regional neuropharmacology	Poster	Wed	1:00 PM
351.	Subcortical organization	Poster	Fri	8:30 AM

Theme I: Neural Basis of Behavior

Session Number	Session Title	Type	Day	Time
28.	Aging and behavior I	Poster	Mon	8:30 AM
29.	Aging and behavior II	Poster	Mon	8:30 AM
358.	Alcohol and barbiturates: biochemistry	Poster	Fri	8:30 AM
359.	Alcohol: behavior	Poster	Fri	8:30 AM
357.	Alcohol: environmental influences and electrophysiology	Poster	Fri	8:30 AM
61.	Angiotensin and drinking	Poster	Mon	1:00 PM
185.	Biological rhythms I	Slide	Wed	8:30 AM
313.	Biological rhythms II	Poster	Thu	1:00 PM
312.	Biological rhythms: suprachiasmatic nucleus	Poster	Thu	1:00 PM
158.	Circuitry and pattern generation I	Poster	Tue	1:00 PM
221.	Circuitry and pattern generation II	Slide	Wed	1:00 PM
299.	Clocks in the test tube: toward a mechanistic analysis of circadian oscillators	**Symp.**	Thu	1:00 PM
47.	Drugs of abuse: nonopiates I	Slide	Mon	1:00 PM
331.	Drugs of abuse: nonopiates II	Poster	Thu	1:00 PM
124.	Effects of chronic drug administration: neurotoxicology	Poster	Tue	8:30 AM
125.	Effects of chronic drug treatment: psychotropics	Poster	Tue	8:30 AM

56.	Feeding and drinking: central mechanisms I	Poster	Mon	1:00 PM
57.	Feeding and drinking: central mechanisms II	Poster	Mon	1:00 PM
58.	Feeding and drinking: central mechanisms III	Poster	Mon	1:00 PM
59.	Feeding and drinking: central mechanisms IV	Poster	Mon	1:00 PM
60.	Feeding and drinking: cues for need state I	Poster	Mon	1:00 PM
62.	Feeding and drinking: cues for need state II	Poster	Mon	1:00 PM
264.	Feeding and drinking: cues for need state III	Slide	Thu	8:30 AM
138.	Feeding and drinking: neuropharmacology	Slide	Tue	1:00 PM
177.	Functional reconstruction of neuronal systems	**Symp.**	Wed	8:30 AM
194.	Human behavioral neurobiology	Poster	Wed	8:30 AM
193.	Human neuropsychology I	Poster	Wed	8:30 AM
269.	Human neuropsychology II	Slide	Thu	8:30 AM
192.	Interhemispheric relations	Poster	Wed	8:30 AM
51.	Invertebrate learning and behavior I	Slide	Mon	1:00 PM
268.	Invertebrate learning and behavior II	Slide	Thu	8:30 AM
12.	Learning and memory: anatomy I	Slide	Mon	8:30 AM
189.	Learning and memory: anatomy II	Poster	Wed	8:30 AM
100.	Learning and memory: anatomy and physiology	Slide	Tue	8:30 AM
191.	Learning and memory: hippocampal physiology	Poster	Wed	8:30 AM
142.	Learning and memory: pharmacology I	Slide	Tue	1:00 PM
239.	Learning and memory: pharmacology II	Poster	Wed	1:00 PM
240.	Learning and memory: pharmacology of conditioning	Poster	Wed	1:00 PM
190.	Learning and memory: physiology	Poster	Wed	8:30 AM
162.	Monoamines and behavior I	Poster	Tue	1:00 PM
163.	Monoamines and behavior II	Poster	Tue	1:00 PM
340.	Monoamines and behavior III	Slide	Fri	8:30 AM
286.	Motivation and emotion	Poster	Thu	8:30 AM
285.	Motivation and emotion: self-stimulation	Poster	Thu	8:30 AM
157.	Neuroethology: avian auditory system and vocalization	Poster	Tue	1:00 PM
154.	Neuroethology: nonavian auditory system and vocalization	Poster	Tue	1:00 PM
156.	Neuroethology: nonteleosts	Poster	Tue	1:00 PM
155.	Neuroethology: teleosts	Poster	Tue	1:00 PM
115.	Neuropeptides and behavior	Poster	Tue	8:30 AM
81.	Neuropeptides and behavior: opiates	Poster	Mon	1:00 PM
63.	Neuropeptides and behavior: vasopressin and oxytocin	Poster	Mon	1:00 PM
80.	Opiates I	Poster	Mon	1:00 PM
82.	Opiates II	Poster	Mon	1:00 PM
261.	Opiates III	Slide	Thu	8:30 AM
126.	Psychotherapeutic drugs: antidepressants	Poster	Tue	8:30 AM
128.	Psychotherapeutic drugs: anxiolytics	Poster	Tue	8:30 AM
127.	Psychotherapeutic drugs: neuroleptics	Poster	Tue	8:30 AM
2.	Sexually dimorphic behaviors: differentiation in mammals	**Symp.**	Mon	8:30 AM
348.	Sleep	Poster	Fri	8:30 AM
161.	Stress, hormones, and the autonomic nervous system I	Poster	Tue	1:00 PM
307.	Stress, hormones, and the autonomic nervous system II	Slide	Thu	1:00 PM
326.	Stress, hormones, and the autonomic nervous system III	Poster	Thu	1:00 PM

223.1 DOES TIMING PLAY A ROLE IN GENERATING ORDER IN THE INITIAL RETINOTECTAL PROJECTION? C. E. Holt* (SPON: W.A. Harris). Dept. of Biology, B-022, Univ. of California San Diego, La Jolla, California 92093.

At the outset of tectal innervation, retinal nerve fibres grow directly to their appropriate target areas to form an ordered visual map in Xenopus laevis (Holt and Harris, Nature 301:150-152, 1983). The question of whether a timing mechanism plays a role in establishing this early map has been examined. First, the normal schedule of axonal outgrowth from the early retina was characterised. Dorsal and ventral thirds of embryonic eyes were labelled by in vitro incubation in ^{3}H-proline (see ref. above). Experimental animals were fixed at each stage of development from the start of retinal axonal outgrowth (stage 28) to early stages of tectal innervation (stage 42). The timecourse and target specificity of axons from these two retinal extremes (dorsal and ventral) were compared autoradiographically. Fibres from dorsal retina were found to grow out of the eye first, along the optic pathway and into the tectum 10-12 hours ahead of ventral fibres. Thus, the tectal rudiment becomes innervated in a specific and sequential manner with the ventral part being innervated first by dorsal retinal fibres (stage 37/38) and the dorsal part second by the later arriving ventral fibres (stage 40).

To reverse this sequence of target innervation and thereby test the importance of temporal factors, heterochronic transplants were done from young to old embryos. Dorsal halves of eyes from stage 21 embryos were ^{3}H-proline labelled and transplanted to stage 27 hosts. Autoradiographs of brains were examined from stage 32 to 42. To control for possible retardation effects, ventral halves of eyes receiving stage 21 transplants were labelled and the timecourse of their fibre outgrowth compared with normal ventral fibre growth.

The heterochronic exchanges retarded dorsal fibre outgrowth by at least 12 hours so that they arrived in the tectum at stages 40 to 41. They had no effect on the timecourse of ventral fibre outgrowth. In most cases, ventral fibres grew out and innervated the tectum before the normally pioneering population of dorsal fibres. Despite this temporal disruption, the results showed that initial tectal innervation was specific: delayed dorsal fibres terminated in ventrolateral tectum while ventral fibres (induced pioneers) went to dorsomedial tectum.

It is concluded that temporal factors do not play a major role in vertebrate neural mapping.

Supported by the McKnight Foundation.

223.2 THE SIMULTANEOUS ELIMINATION OF IMPULSE ACTIVITY AND NORMAL PATHWAYS DOES NOT PREVENT DEVELOPING RETINAL AXONS FROM REACHING THEIR NORMAL TARGETS. W. A. Harris. (Sponsor: F. Denaro) Department of Biology, B-022, University of California, San Diego, La Jolla, California 92093.

Retinal axons can grow to their appropriate targets along unusual pathways, as demonstrated by tracing the retino-tectal projections of eyes embryonically transplanted to genetically eyeless axolotls, (Harris, J.C.N. 194:303-317, 1980). They can also find their correct targets in the absence of impulse activity, as shown by studying the optic pathways from axolotl eyes embryonically transplanted to the TTX-harboring California newt (Harris, J. Neurosci. 2:339-353, 1982). In the present experiments these two factors were simultaneously eliminated. Retinal axons were challenged to grow along abnormal pathways in the absence of impulse activity.

Eye primorida were first transplanted from normal to eyeless axolotl embryos, making sure to graft the optic stalk onto an ectopic site in the host brain. These axolotl embryos were then parabiotically joined to California newt embryos. Both operations were completed by stage 28, i.e. before axons had left the eye. The result of the parabiosis was a paralysis of the "eyeless" axolotl twin due to the newt's TTX. The newt twin remained normally active. The pair was allowed to develop for about one week. When the axolotl twin reached approximately stage 44, a needle coated with HRP was jabbed into either the dorsal or the ventral part of the silent transplanted retina. After 2 days the animals were killed, fixed, cryostat sectioned, and reacted. The results from 21 such cases show normal projections: the dorsal retina to the ventrolateral tectum, and the ventral retina to the dorsomedial tectum. Unusual pathways were often taken to achieve these destinations. Control animals, both normal axolotls developing alone and normal axoltls parabiosed to newts, showed the same patterns. These results indicate that the retinal projections in the experimental group were basically normal.

Thus, fibers need neither impulse activity nor a particular pathway to navigate to their correct targets during development. Both factors can be eliminated simultaneously, yet retinal axons still find their way to the tectum and make an ordered map. Other experiments from our laboratory have ruled out timing mechanisms (Holt, above), and fiber-fiber interactions, the latter also in combination with the elimination of impulse activity (Ferguson, below) as crucial factors in the formation of the early retinotectal projection. Clearly, other sorts of cues must be more critical for axonal navigation in this system.

223.3 SIMULTANEOUS ELIMINATION OF NORMAL FIBER-FIBER INTERACTIONS AND IMPULSE ACTIVITY DOES NOT PREVENT APPROPRIATE TECTAL INNERVATION. Betty Alice Ferguson, Department of Biology, B-022, University of California, San Diego, La Jolla, Calif. 92093.

Retinal axons form a visual map upon first innervating the tectum during normal development in Xenopus (Holt and Harris, Nature 301:150-152, 1983). Mechanisms proposed to explain appropriate innervation patterns include (among others) timed outgrowth, activity dependent interactions, pathway selection, and fiber-fiber interactions. Temporal factors alone do not determine the pattern of innervation (Holt, above). In addition, axolotl retinal axons grow to appropriate targets along abnormal pathways in combination with TTX-blocked activity (Harris, above). This experiment tests the affect of simultaneously eliminating normal fiber-fiber interactions and Na^{+}-dependent impulse activity of the specificity of retinal-tectal innervation.

Embryonic eyes were removed from stage 25-27 Xenopus (prior to axon outgrowth), the dorsal (D) or ventral (V) third incubated in ^{3}H-proline (Holt and Harris, ref. above) and only the labelled third replaced in the embryo. The embryos were allowed to heal a few hours, then parabiosed to TTX-producing newt embryos, Tari cha torosa, and allowed to develop to stage 40-46. The parabiosis results in paralysis of the Xenopus embryo; the newt embryo is active as normal.

Control experiments (in collaboration with C. Holt) show that labelled D or V thirds stage 40-46 in nonparabiosed embryos grow to the appropriate tectal region (D; 21/23 appropriate; V 19/20 appropriate). In the 38 stage 44-46 parabiosed embryos which have tectal projections, 10/19 D eye projections were appropriate, 15/19 V eye projections were appropriate. The eye pieces in the remaining embryos expanded somewhat into inappropriate tectal areas; this occurred more at later stages.

Thus, despite disruption of normal fiber-fiber interactions and block of Na^{+}-impulse activity, retinal ganglion cells can grow to the tectum and project initially to appropriate areas; D eye to ventrolateral tectum, V eye to dorsomedial tectum. There may be an expansion later in development to uninnervated regions of the tectum.

Supported by the NIH.

223.4 DEVELOPMENT OF VISUAL PATHWAYS IN XENOPUS LAEVIS: AN AUTORADIOGRAPHIC ANALYSIS. P. Grant and P.M. Ma*. Dept. of Biology, University of Oregon, Eugene, Oregon 97403.

Continuing our studies of the rules governing optic fiber connectivity, we have completed an autoradiographic analysis of development of the optic fiber pathway in Xenopus. ^{3}H Proline (3×10^{-5}-1.0 µL) was injected into the eyes of embryos and larvae at different stages through metamorphosis. After 4 hrs. to 5 days (depending on stage), whole embryos, tadpole heads or isolated brains were fixed in Bouins, dehydrated, embedded in paraffin and serially sectioned at 10µ in three planes. Slides were dipped in Kodak NTB2 emulsion, dried and exposed for two weeks, developed in Kodak D19, counterstained with cresyl violet and mounted. Autoradiographs were photographed or drawn with camera lucida and serial section reconstructions prepared.

Optic fibers first arrive at the di-mesencephalic junction at stage 36/38. Some turn caudally, entering ventral, rostral and lateral mesencephalon (tectum), reaching dorsal tectum by stage 39/40. During development, fibers continue to extend progressively over the growing tectum with rostrocaudal, ventrodorsal and lateromedial trajectories, patterns correlating directly with the known order of tectal maturation. Meanwhile, other labeled fibers turn rostrally from the di-mesencephalic border to form pretectal and putative uncinate neuropils. Later, during stages 42-50, labeled fibers accumulate caudorostrally, first in the Bellonci neuropil (NB), then in the corpus geniculatum thalamicum and finally in the rostral visual nucleus, a temporal ordering corresponding to the caudorostral sequence of diencephalic maturation. The most ventral fibers at the di-mesencephalic border turn caudally at stage 38 beneath the mesencephalon and form the basal optic tract and neuropil (BON). As the BON enlarges during development, neuropil layers appear at stage 48-50, forming a dorsal-ventral pattern resembling the juvenile BON.

The first fibers of the ipsilateral diencephalic projection appear at stage 56/57, running up the lateral edge of the diencephalon. Fibers terminate in all diencephalic neuropils at about the same time. The ipsilateral NB, in contrast to the contralateral NB, is layered from the outset, with fibers terminating in several small peripheral regions separated by non-innervated gaps. Later, layering becomes more complex as contra- and ipsilateral projections segregate within the NB.

During development, most optic fibers enter the tectal neuropil along a rostrocaudal trajectory while others follow the opposite trajectory, entering diencephalic neuropils along a caudorostral trajectory. These patterns correlate with the temporal order of post synaptic target cell maturation and are consistent with a spatio-temporal model for the ordering of optic fiber connectivity.

223.5 HOW RETINA AND TECTUM GROW IN POSTEMBRYONIC GOLDFISH. Pamela A. Raymond. Department of Anatomy & Cell Biology, Univ. of Michigan Medical School, Ann Arbor, MI 48109.

In teleost fish throughout postembryonic life optic fibers from the retina grow into the optic tectum, where they form a retinotopic array of synaptic connections. Using ^{3}H-thymidine radioautography to label dividing cells and computer-aided graphics to reconstruct 3D shapes, I have charted the growth of retina and tectum in goldfish from hatching through adult life, to test the hypothesis that retinotectal connections change as a consequence of a mismatch in the patterns of growth.

In adult goldfish, both retina and tectum approximate a hemisphere, although the tectum is markedly lopsided, bulging forward rostrally and becoming almost planar caudally. In larval and young juvenile fish, up to about 80 days of age, the tectum is symmetric and somewhat flattened. Both retina and tectum enlarge by two mechanisms: 1) stretching of existing tissue and 2) addition of new neurons to the rim of the hemisphere. From hatching onward, the retinal germinal zone is circumferential; thus annuli of new optic fibers are added with growth. Addition of new tissue is at first equivalent at dorsal and ventral margins, but during the transition between larval and juvenile stages, ventral begins to lag behind dorsal (increments added ventrally are about 25% shorter). Growth by stretching is most prominent in peripheral retina: in one fish the central 25% of the retina increased 15% in area, whereas the most peripheral annulus, encompassing 10% of the retina, increased 40% in area over a 230 day period.

The tectal germinal zone is U-shaped at all post-embryonic ages. Germinal cells are absent from about 1/3 of the rim at the rostral pole, so that only partial annuli of new tectal cells are added. The gap in the tectal germinal zone is topographically equivalent to temporal retina. The consequences of this pattern of tectal cell addition are: 1) the asymmetric curvature of the adult tectal surface is generated and 2) the earliest formed part of the tectum is at the rostral pole. Thus in fish injected with ^{3}H-thymidine at larval stages and sacrificed as adults, a partial annulus of labeled cells abuts the rostral tectal pole and encloses a region, less than 10% of the total tectal surface area, which represents the larval tectum. An annulus of label in the retina encloses a region of similar relative area, but of different topographic location, in central retina, surrounding the optic disk. Because central retina always projects to central tectum, optic fibers derived from the larval retina must project to a region of the adult tectum which was not generated until postlarval life, and which is up to 1 mm away from the original site of termination of the larval fibers. (Supported by EY04318).

223.6 PLASTICITY IN THE RETINOTECTAL PROJECTION DURING NORMAL DEVELOPMENT: SLIDING CONNECTIONS IN RANA. S. E. Fraser. Department of Physiology & Biophysics, University of California, Irvine, CA 92717.

The retinotectal system of lower vertebrates shows a great degree of plasticity following experimental perturbations of either the retina or the tectum. Gaze and his coworkers realized that a similar plasticity may play a role in normal development because: (i) the retinotectal projection is ordered normally during development, (ii) the retina and tectum grow throughout development, and (iii) the patterns of growth do not match. They proposed that to compensate for this mismatch of growth patterns, the retinotectal projection must continually shift during development. Experimental evidence for this shift has been obtained in Xenopus (anatomical: Gaze, et al JEEM 53:103; physiological: Fraser J.Physiol. 305:113p; Dev. Biol. 95:105). However, these studies were limited by the small size and poor optics of the Xenopus visual system and the asymmetric growth of the Xenopus retina that may in part compensate for the growth mismatch.

Here I report on an extension of these results to a preparation that avoids these limitations: the Rana pipiens retinotectal projection. Light-pipe mapping of the retinotectal projection (Fraser J.Physiol 305:113p) was used to directly assay the region of the tectum at which the retina surrounding the optic nerve head terminated. Following this determination, a small electrolytic marker-lesion was placed at that location in the tectum. The animal was revived and allowed to develop for several days at which time the procedure was repeated. The interlesion distance should therefore yield a direct measure of the shift in the retinotectal projection during the inter-lesion time period.

Following fixation, the animals were imbedded in paraffin, sectioned at 10um and stained by the picro/panceau technique. The marker lesions appear as small darkened regions of cellular debris that persist for up to two weeks. Measurements of the lesion positions were made by counting the sections between lesion (anterior-posterior distance) and measuring the distance from the midline of the tectum to the lesion (medial-lateral distance).

Thirty Stage XIX animals were subjected to the double lesion protocol, of which nine lived and six were interpretable. The average shift in the position of the central retina was 275um ±80um (S.D.) from Stage XIX to Stage XXI (movement in the posterior-medial direction). This rate of shift obtained for Rana is at least double that observed in previous experiments on Xenopus and is consistent with the anatomical results recently obtained by Reh & Constantine-Paton (personal communication).

223.7 EYE-SPECIFIC STRIPES IN THE TECTAL LOBES OF THREE-EYED FROGS ARE DEPENDENT ON NEURAL ACTIVITY. M. Constantine-Paton and T. Reh, Biology Department Princeton University, Princeton NJ 08544.

Three-eyed Rana pipiens, produced by adding a third eye primordium to the early embryo invariably develop highly periodic eye-specific segregation in tectal lobes dually innervated by a host and the supernumerary optic tract. We have suggested that these stripes arise as a compromise between two mechanisms normally involved in producing the single retinotectal map: one which is dependent on cell surface affinities and aligns the afferent projection(s) within the tectum and a second that produces segregation and increases point to point order by maintaining as neighbors in the tectal lobe only terminals that arise from neighboring ganglion cells in the retina. We have now tested the idea that this second segregating mechanism is dependent on neural activity by blocking Na^+-dependent spikes in all optic nerves of 3-eyed tadpoles. Segregation was assessed by HRP histochemistry followed by autoradiography after labeling supernumerary projections with HRP and both normal eyes with ^{3}H-proline.

TTX was applied either in the vitreous or behind the eyes of tadpoles in a slow release plastic which assured continuous presence of low concentrations of the drug(10^{-6}-$^{-8}$M)for up to four weeks. Effective blocking doses were determined by recording in the tectal lobe and continued blockade of retinal input to the brain was assessed by using the optokinetic turning response of tadpoles. We established that 2 weeks after crushing the normal optic nerves of 3-eyed tadpoles the supernumerary retinal projection became continuous. By four weeks, the normal eye's projection regenerates to the tectum and covered most of it. By 6-7 weeks post-optic nerve crush (ONC) the periodic pattern of eye-specific segregation was once again pronounced. TTX blockade during the last three weeks of this process eliminated resegregation.

We have also subjected three-eyed tadpoles without ONC to TTX blockade for 2, 3 and 4 weeks. Animals sacrificed at two weeks show overlap in the rostral tectum but distinct interdigitating stripes in other regions of these lobes. After three weeks of blockade however, HRP labeled terminals in more central regions of the doubly innervated lobe began to invade stripes previously dominated by the host retina. After 4 weeks of blockade these terminals had spread to form a continuous projection throughout the doubly innervated tectal lobe.

We conclude therefore that action potential activity is crucial both to the development and to the maintenance of eye-specific segregation in the tecta of 3-eyed tadpoles. Supported by NIH Grants EY01872 and EY05579.

223.8 PEPTIDE-LIKE IMMUNOREACTIVITY IN REGENERATING ANURAN OPTIC NERVE FIBERS. Rodrigo O. Kuljis and Harvey J. Karten. Depts. of Neurology, Psychiatry and Neurobiology, S.U.N.Y. at Stony Brook, N.Y. 11794.

Peptide-like immunoreactivity (PLI) Has been shown in various classes of amacrine cells in vertebrate retinae. Ganglion cell layer elements, however, have been consistently PLI-negative (Brecha, Karten & Laverack, '79, Karten, Reiner & Brecha, '82). Interestingly, an elaborate pattern of PLI exists in the retino-recipient portion of the anuran optic tectum (Kuljis & Karten, '81 & '82c). This pattern is dramatically modified by retinal deafferentation, thus suggesting transsynpatic effects and/or the loss of PLI-containing retinal terminals (Kuljis & Karten, '82 & '83a). Furthermore, optic nerve ligation is followed by the appearance of PLI in the retinal stump of nerve fibers that suggests a post-traumatic response and/or the presence of normally non-demonstrable peptides in retinal ganglion cells (Kuljis & Karten, '82b & '83b). The present report further investigates the possibility of the presence of peptides in retinal ganglion cells by means of regeneration techniques.

Twelve specimens of Rana pipiens were subjected to unilateral optic nerve crushing. One hour to 120 days after surgery, substance P-, leucine enkephalin-, cholecystokinin octapeptide - and bombesin-like immunoreactivities were analyzed in the retinae and optic nerves by means of peroxidase-anti-peroxidase and fluorescence methods.

Retinal ganglion cell bodies failed to display unequivocal PLI throughout the whole postoperative period. PLI was observed in fine longitudinally oriented beaded fibers, often ending in an expansion directed towards the brain. During the first 2-3 months following the operation most of the fibers accumulated in the retinal stump of the optic nerve. Two to 3 months after surgery, some of the fibers traversed the injury site towards the brain, mainly on the periphery of the nerve, and fascicles of the fibers invaded the cerebral stump of the optic nerve. Some fibers reached the optic chiasm. Experiments are in progress to analyze the reinnervation of the tectum.

These observations support the possibility that retinal ganglion cells contribute to PLI in the tectum. However, the possibility that PLI present in regenerating optic nerve fibers is a post-traumatic response, not reflecting a normal feature of retinal ganglion cells, cannot be dismissed. Supported by 1-F05-TW02947 (R.O.K.) & EY04796 (H.J.K.)

223.9 THE SUBSTRATE FOR OPTIC NERVE FIBRE GROWTH IN FISH. J.H.Scholes*and A.Maggs (SPON: M.Djamgoz). MRC Cell Biophysics Unit, King's College London, 26-29 Drury Lane, London WC 2. England.

Optic nerves in fish tend to be sheet-like, forming elaborate pleated patterns during post-embryonic growth (Scholes, J.H., Nature 278: 620, 1979). This arises because new retinal axons extend toward the brain throughout life in a narrow (20-30μm) growth tract at one edge of the nerve.

Seen by electron microscopy, the substrate encountered by growing fibres in the optic nerve has various unusual features which may underlie their tightly focussed tracking behaviour and the resultant pattern developed in the entire optic nerve. These are (1) that satellite cells of all kinds, and their processes, are excluded by the growing fibre bundle, and (2) that optic nerve fibre growth cones form a continuous queue, several wide, occupying a narrow track on the basal lamina surrounding the nerve. In growing animals, this matrix track has a complex reactive form, differentiated from the rest of the basal lamina at the surface of the optic nerve and CNS, which is populated to confluence by astrocytic end-feet.

Maintenance of this specialised substrate apposing growing fibres can be probed with labelled amino acids (particularly PRO) injected into the eye in less than endogenous concentrations. They enter the rapid transport mechanism of growing fibres specifically, arriving in the growth track in free, detergent accessible form. As seen by ARG of sections and of SDS-PAGE, they become available there for local synthesis of matrix proteins, particularly collagen.

223.10 PERSISTENCE OF A NORMALLY-TRANSIENT PATTERN OF SUPERFICIAL OPTIC AXON FASCICLES IN THE SUPERIOR COLLICULUS OF REELER MUTANT MICE M.A. Edwards and V.S. Caviness, Jr. E.K. Shriver Center, Waltham, Ma. 02154

We previously reported (Neurosci. Abst. 7:733,'81) that fascicles of optic fibers in normal mice initially course through the presumptive superficial grey stratum (SGS) of the superior colliculus (SC) but dissappear by an unknown mechanism by the end of the first postnatal week. In the adult reeler mutant mouse many optic axons have anomalous trajectories through the SGS rather than the underlying stratum opticum (SO), as in the normal (Frost et al, Neurosci. Abst. 8:821,'82). The developmental origin of the abnormal stratification in reeler is the subject of the present investigation.

Only subtle abnormalities of cell pattern are detected in the reeler tectum. These include a somewhat disorderly arrangement of superficial postmigratory cells, evident by embryonic day 16 (E16), and an apparent reduction in the width of the stratum marginale, apparent after postnatal day 1 (P1). The pattern of histogenesis of the visual layers of SC, as reflected in the positions of successively generated neuronal cohorts labeled with tritiated thymidine, is indistinguishable in the two genotypes. By E17, many cells heavily labeled by injections on E11 and E13 attain an overlapping distribution in the superficial layers of SC in normal and mutant animals alike. In both genotypes the initial retinal projection, as defined by anterogradely transported HRP and normal fiber impregnations on E15-16, consists of only superficial fiber bundles. By the day of birth (P0), the distribution of longitudinally coursing fiber bundles within presumptive SO and SGS is the same in both reeler and normal mice. In the normal mouse superficial fiber fascicles diminish progressively after P2 and are absent after P6; in the reeler by contrast, up to 50% of the bundles persist. The retinal origin of the fiber fascicles present in the normal SGS and upper SO is verified by their rapid disappearance following bilateral eye removal. After P10 the total number of fiber fascicles in both SO and SGS in the mutant is similar to that of SO in the normal, suggesting that there is no hyper-innervation in reeler SC. Thus it appears that a pattern of projection which is transient in the normal persists in the reeler mouse. It is uncertain whether the abnormal axons present at maturity in the mutant SGS correspond to those that are lost in the normal animal. Alternatively, many may be late-arriving fibers guided incorrectly to SGS instead of SO along a smaller number of abnormally persisting bundles. (Reseach supported in part by NIH grants NS 12005 and EYO 4549).

223.11 HEALING AND GROWTH PATTERNS AFFECT THE VISUOTECTAL PROJECTIONS IN EYE FRAGMENTS AND CHIMERIC EYES. C. F. Ide*, P. Reynolds*, C. Falk*, D. Kahn*, B. Szaro*, D. Reinschmidt* and R. Tompkins. Dept. of Biology, Tulane University, New Orleans, LA 70118.

The normal growth pattern of embryonic Xenopus eyes - basin cells withdraw from mitosis early and all new cells are added in annular rings from the ciliary margin - has heretofore been assumed to follow surgical ablation and chimeric eye construction. Hence, mirror image duplications resulting from one-third size nasal or temporal fragments as well as normal visuotectal projections resulting from heterotopic graft chimeric eyes suggested that regulative changes in positional information had occurred. Our data show that healing and growth patterns following such surgery are not normal and that specific healing patterns correlate with the visuotectal projections formed. Nasal and temporal one-third eye fragments, formed by ablation at stage 32-33 heal and, in about 50% of the cases, survive to make eyes in the postmetamorphic animals which have mapable visuotectal projections. Most nasal one-third eyes and a minority of temporal fragments heal by the extrusion of cells from the center of the cut edge into the region of the ablation, forming a tongue of cells between the distal cut edges. These tiny anatomical duplicates develop into eyes with duplicated visuotectal projections. Most temporal one-third fragments and a minority of nasal fragments heal by rounding up; that is, the distal cut edges collapse to meet in the region of the ablation. This healing pattern is correlated with the formation of unduplicated visuotectal projections. During tongue formation, neurons and undifferentiated cells are transferred from the original fragment into the tongue in a disorderly array, but quickly sort out to reform normal retinal architecture. We postulate that the tongue cells retain their original determination to connect to the same tectal positions as the fragment from which they originate, despite their new positions, and that this mosaicism, coupled with cell movement into the tongue, establishes duplicate visuotectal projections. Heterotopic dorsal into antero-ventral grafts, performed at stage 32-33 using tetraploid cells to mark the graft, sometimes form mosaic visuotectal projections. In many cases, marked graft cells are found in the dorsal portion of the eye, contrary to expectation. The demonstration that retinal neurons can move, sort out, and, in the case of heterotopic graft chimeric eyes, come to reside in their region of origin during healing and early growth of surgically perturbed eyes must force a re-evaluation of studies of surgically perturbed eyes where the assumption of normal growth leads to the conclusion that regulative alterations of positional information have occurred. (Supported by NSF grant BNS-8216681)

224.1 INTERACTIONS BETWEEN CHEMICAL AND ELECTRICAL KINDLING: C.G. Wasterlain* and D. Fairchild* (SPON: I. Gerson). Epilepsy Research Lab, VAMC, Sepulveda, CA. 91343, Dept. of Neurology and Brain Research Institute, UCLA School of Medicine.
Previous work in our laboratory has suggested that the genesis of the kindled focus involves a great deal of both neurochemical and neuroanatomical specificity. The current study reinvestigates the interaction between chemical and electrical kindling in two anatomical locations: the amygdaloid region and the septal-hippocampal complex. The amygdala was implanted with chemitrodes in which the tips of the bipolar stainless steel electrode and of the injection cannula were closely approximated. For septal-hippocampal kindling, the stimulating electrode was implanted high in the medial septum , the cannula in the dorsal hippocampus. Medial septal stimulation resulted in prominent hippocampal afterdischarges, which were abolished by transsecting the fimbria, suggesting mediation through the septal-hippocampal excitatory pathway which provides over 90% of the muscarinic innervation of the hippocampus. Half the animals were kindled electrically, and one day after kindling (three stage 5 seizures) was obtained, chemical kindling was begun. The other half was kindled chemically first, then electrically. The results differed with the anatomical location. With amygdaloid implants, the rate of electrical kindling of naive animals did not differ significantly from that of rats which had first been fully kindled chemically. Similarly, the rate of chemical kindling did not show a significant dependence upon a previous electrical kindling experience. However, in both cases a non-significant trend toward positive transfer was observed. In the septal-hippocampal group, by contrast, significantinteractions were observed in both directions. Rats previously kindled by electrical medial septal stimulation displayed accelerated carbachol hippocampal kindling compared to their naive counterparts. Similarly, kindling by electrical septal stimulation was faster in rats previously kindled by repeated carbachol injections into dorsal hippocampus than in a naive group. These results suggest that chemical and electrical kindling involve similar mechanisms, and that the extent to which transfer occurs reflects the degree to which they share a common chemical anatomy. We hypothesize that carbachol kindling of the hippocampus and electrical kindling of the medial septum share a common excitatory muscarinic stimulation of hippocampal neurons, and that the site(s) of interaction may be in part located in these neurons or in their projections. Supported by the Research Service of the Veterans Administration.

224.2 **GLUTAMERGIC AFFERENTS FROM PYRIFORM CORTEX TO AMYGDALA ARE INVOLVED IN ELECTRICAL KINDLING OF THE RAT.** **Isaac L. Crawford, Jonathan E. Walker, Richard W. Homan, and Maria Barletta*. Departments of Neurology and Pharmacology, UTHSC, Southwestern Medical School and VA Regional Epilepsy Center, Dallas, TX 75216**

We designed studies to determine whether glutamate may be involved in kindling of the amygdala in rats. Stainless steel bipolar electrodes were stereotaxically implanted in the left basolateral amygdala of experimental and control rats; the left pyriform cortex was ablated, while control rats were sham operated. Daily stimulation (400 uA, 1 sec, 60 Hz) began one week later. Rats were sacrificed 24 hours after their third consecutive stage 5 seizure. Pyriform ablation markedly prolonged the time to kindling compared to controls (39 ± 6 vs 11 ± 1 day, $p<0.001$). In the amygdala of kindled sham operated rats high affinity glutamate uptake was not significantly different from that in the contralateral amygdala of the same animals (27.5 ± 3.4 vs 21.7 ± 2.1 umol/hr/g wet weight). In contrast, removal of the pyriform cortex reduced high affinity glutamate uptake to less than half that in the amygdala of the unoperated side.

In a second series of experiments, rats were kindled and then the pyriform cortex was ablated. Sham operated controls rekindled within 2 days, while experimental rats required 7 ± 2 days to regain stage 5 seizures. These findings indicate the potential importance of a glutamergic input in the development of the kindling process and its less critical role in the persistence of kindling. The results also suggest that the influence of glutamergic afferents may be largely postsynaptic.

Supported in part by VA Merit Review Research Programs.

224.3 GLUTAMIC ACID: THE ROLE OF A NEUROTRANSMITTER IN THE FACILITATION OF KINDLING AND THE DEGENERATIVE DISORDERS OF THE CNS, M. Girgis and K. Harris*. Dept. of Anatomy, Univ. of Sydney, N.S.W., Aust.

The prolonged depolarizing effect, with subsequent lowering of threshold of stimulation that would be associated with an abnormal accumulation of excessive quantities of glutamate, could indicate a causal role of glutamate as a trigger factor in epileptic discharge. By direct application of exogenous glutamate, we have been able to successfully chemically kindle the amygdala obtaining stage 4 seizures. Intracerebral implantation of 'chemitrodes' was done in limbic structures of cat brains. In some experiments, repeated injections were made by a microsyringe. In others, steady-state flow of glutamate was produced by means of an osmotic minipump lasting one to two weeks. In some experiments, we used kainic acid in minimal doses prior to the glutamate minipump, and this catalyzing the reaction, acted as a primer in the production of the original (pathological) locus as found in the human disorder. Stage 6 seizure was observed in these preparations.

On perfusion, degenerative changes were observed in the brains of the glutamate preparations (these were greatly pronounced in the kainate experiments). Histologically, sclerotic plaques were seen diffusely in different brain areas in both preparations. EEG recordings showed very high amplitude spike activity at the site of injection, which later spread to other brain areas.

Similar EEG results were found in earlier experiments where cholinergic agents were used for producing animal models of limbic epilepsy (Girgis, M., Neurosci., 6:1965, 1981). As stimulation of acetylcholine receptors results in sustained seizure activity which damages the brain by unknown mechanisms, and as endogenous glutamate has possible excitotoxic action, it seems likely that glutamate may have a role in the brain damage associated with sustained limbic seizures.

Recent studies support the theory of glutamate as an endogenous neurotoxin. (Brookes, N., et al, Soc. for Neurosci. Abst., 23:15, 1982). By extrapolation, glutamate is therefore capable, via its necrotizing effect, of producing the epileptic focus associated with limbic epilepsy. The feasability of glutamate or glutamate-using systems being implicated in neurodegenerative disorders, as yet of unknown etiology, is also under investigation.

224.4 BENZODIAZEPINE-RELATED MECHANISMS IN THE AMYGDALOID KINDLING MODEL OF EPILEPSY IN THE RAT. W. S. Schwark, M. Haluska* and P. Blackshear*, Department of Pharmacology, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.

Neuropharmacological and neurochemical investigations of the involvement of the benzodiazepine-GABA receptor-chloride ionophore complex in the seizure state induced by amygdaloid kindling were undertaken in adult male rats. Intraperitoneal diazepam markedly inhibited seizure intensity and afterdischarge duration in this seizure model, with an ED50 of approximately 2 mg/kg. The inhibitory potency of a series of benzodiazepines (including medazepam, diazepam, clonazepam and nitrazepam) on amygdaloid kindled seizures approximately paralleled their depressant effect on maximal electroshock-induced seizures and their Ki for [^{3}H]-diazepam binding in rat brain membranes. Intraventricular administration of inosine (150 µg), a putative endogenous benzodiazepine receptor ligand, significantly increased seizure latency and significantly decreased afterdischarge duration. Intraperitoneal muscimol (1 mg/kg), a GABA receptor agonist, also produced a significant prolongation of seizure latency.

In vitro binding studies with [^{3}H]-diazepam in brain membranes obtained from normal rats indicated that two populations of benzodiazepine receptors exist in brain regions such as the frontal cortex, i.e., a high affinity population (KD = 6.3 ± 1.2 nM; Bmax = 0.79 ± 0.19 pmol/mg protein) and a low affinity population (KD = 28.2 ± 4.9 nM; Bmax = 1.71 ± 0.27 pmol/mg protein). In contrast, a single benzodiazepine receptor type was found in the cerebellum (KD = 6.8 ± 0.8 nM; Bmax = 0.85 ± 0.08 pmol/mg protein). [^{3}H]-Diazepam binding properties were not altered in the cerebellum or frontal cortex of kindled rats which had not experienced a seizure within 1 week prior to the time of sacrifice. However, significant increases in cerebellar KD and Bmax values were observed in kindled rats which experienced a seizure just prior to death. In addition, inosine-induced increases in KD of [^{3}H]-diazepam binding were markedly impaired in cerebellar tissues obtained from kindled rats. Our data support the idea that alterations in endogenous mechanisms mediated by the benzodiazepine receptor complex may be instrumental in the epileptogenesis produced by amydgaloid kindling.

Supported by the Epilepsy Foundation of America.

224.5 INTERACTIONS OF CARBAMAZEPINE WITH BENZODIAZEPINE SYSTEMS IN AMYGDALA KINDLING. Susan R.B. Weiss* and Robert M. Post. Biological Psychiatry Branch, NIMH, Bethesda, MD 20205.

The mechanism of action of carbamazepine, an anticonvulsant with efficacy in manic-depressive illness (Post, Psychol. Med. 12:701-704, 1982), is not known. Marangos et al. (Eur. J. Pharmacol., 1983, in press) observed that carbamazepine weakly displaced ligands binding at the central benzodiazepine site and more potently displaced [^{3}H] Ro 5-4864 which binds to the "peripheral" site. We examined whether carbamazepine's effects on benzodiazepine receptor systems are related to its anticonvulsant efficacy.

Rats, implanted with electrodes in the amygdala, were stimulated once-daily until major motor seizures were reliably produced. The anticonvulsant effects of carbamazepine (10 and 15 mg/kg) were studied following pretreatment with vehicle or the relatively pure antagonists of the central benzodiazepine binding site (Ro-15-1788 and CGS-8216). A ligand acting at the "peripheral" benzodiazepine binding site (Ro 5-4864) was also used. Pretreatment with the antagonist Ro-15-1788 (5 and 10 mg/kg), which significantly reverses the anticonvulsant effects of diazepam, had no effect on carbamazepine's ability to inhibit kindled seizures or afterdischarge duration. The antagonist CGS-8216 (5 and 10 mg/kg) was also ineffective in blocking carbamazepine's anticonvulsant effects. In contrast, the "peripheral" benzodiazepine ligand Ro 5-4864 (5 mg/kg) inhibited the anticonvulsant effects of carbamazepine on amygdala-kindled seizures. Ro 5-4864 alone at this dose had little effect on the amygdala-kindled seizure or afterdischarge duration. These results, consistent with the binding data, suggest that carbamazepine's anticonvulsant effects on amygdala-kindled seizures are probably not mediated by a direct effect at the central benzodiazepine receptor site. Possible effects of carbamazepine at the so-called "peripheral" benzodiazepine site are suggested by these data and require further exploration.

224.6 KINDLED SEIZURES ARE PREVENTED BY A BENZODIAZEPINE ANTAGONIST, Ro15-1788:EVIDENCE FOR MULTIPLE BENZODIAZEPINE RECEPTORS. M.L. Riives* and H. A. Robertson, Dept. of Pharmacology, Dalhousie University, Halifax, N. S. Canada B3H 4H7.

In many tests, an imidazodiazepine (Ro15-1788) blocks sedative, anxiolytic and anticonvulsant actions of benzodiazepines such as diazepam. At the same time Ro15-1788 is apparently devoid of intrinsic activity. We used Ro15-1788 to test the hypothesis that an endogenous benzodiazepine-like compound might be working to inhibit the development of amygdaloid kindled seizures. Rats were injected with vehicle, Ro15-1788 (1 or 10 mg/kg, i.p.), diazepam (3 mg/kg) or Ro15-1788 plus diazepam 10 min. before the daily kindling stimulus.

Surprisingly, both Ro15-1788 and diazepam retarded the development of kindled seizures. Most significantly, however, Ro15-1788 completely abolished the sedative-ataxic effects of diazepam (as measured by a vertical screen test) but did not affect the anticonvulsant effects of diazepam.

Because the anticonvulsant effects of Ro15-1788 were antagonized by another benzodiazepine antagonist (CGS 8216), we can presume that the anticonvulsant effects of Ro15-1788 are mediated by a benzodiazepine receptor.

These results suggest that Ro15-1788 is a partial agonist at an anticonvulsant benzodiazepine receptor but is a pure antagonist at the sedative receptor. This supports the idea of distinct and separate benzodiazepine receptors for sedative and anticonvulsant actions.

(Supported by the Savoy Foundation and a Dalhousie Research Foundation Studentship to M.L.R.)

224.7 Hippocampal Kindling: Peptide and Adrenal Hormone Modulation of After-Discharge and Behavioral Depression, G.A. Cottrell, C. Nyakas*, R. de Kloet*, B. Bohus* and D. de Wied*. Rudolf Magnus Institute for Pharmacology, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands.

Hormones can play a role in seizure behavior, at least a permissive role, since ß-endorphin, Leu- or Met-enkephalin produce after-discharge (AD) activity and convulsions and corticosteroids affect the convulsive threshold. We were interested in the effect of hormone manipulation on established seizure behavior. Hippocampal-induced seizures were of special interest since this structure has been implicated in epilepsy and is also part of the neuroendocrine system.

Using hippocampal kindling, a reproducible method for generating characteristic seizures, we have examined the effects of ACTH-like peptides and adrenal steroids on AD and the subsequent behavioral depression (BD). Male wistar rats were kindled daily (1 sec, 60 Hz biphasic square wave, 1 msec pulse duration at threshold AD-eliciting intensity) through 1 bipolar dorsal hippocampal electrode. EEG was recorded from the contralateral hippocampus.

In Experimental Series 1, kindled rats were injected subcutaneously with either peptide or saline 1 hr before the kindling session. Saline was injected between peptide tests until AD and BD values returned to normal. The duration of the AD and BD was reduced jointly or individually by several ACTH-like fragments (ACTH (1-16), ACTH (7-16), [D-Phe7]ACTH (7-16), ACTH (4-16), αMSH vs ACTH (1-16), [D-Phe7]ACTH (7-16), ACTH (4-7), [D-Phe7]ACTH (4-10), ACTH (4-16), resp.) and by γ2-MSH. This indicates that the structural requirements are different for the effects on these two variables.

In Experimental Series 2, the rats were adrenalectomized (ADX) or sham-operated under ether. There was a complex series of changes in AD and BD during the first 24 hrs after ADX, culminating at Day 1 in markedly decreased AD and BD, which returned to normal over the next several days. These changes were normalized after replacement of the ADX group with low doses of corticosterone (0.5 mg/kg s.c.).

Thus, we observed that opiocortin fragments and pituitary-adrenal hormones are important in the expression and maintenance of hippocampal kindled seizures. These findings reinforce the notion that the hippocampus is a neuroendocrine integrator which functions both as a controller of pituitary function and as a target organ for peptide and steroid hormones.

224.8 POTENTIATION OF ARGININE VASOPRESSIN'S CONVULSIVE EFFECTS BY SOMATOSTATIN. D.M. Burnard, Q.J. Pittman and W.L. Veale, Departments of Medical Physiology and Pharmacology, University of Calgary, Calgary, Alberta T2N 4N1.

Both arginine vasopressin (AVP) and somatostatin (SS) have convulsive effects when administered into a lateral cerebral ventricle of the rat (Cohn & Cohn, Brain Res. 96: 138, 1975; Kasting et al., Can. J. Physiol. Pharmacol. 58: 316, 1980). The first exposure to AVP increases the likelihood of a convulsion upon subsequent administration, but no such sensitization process has been reported for SS. The present work sought to determine whether SS and AVP would cross-sensitize, whereby exposure to one convulsive agent lowers the convulsive threshold and potentiates the response to the other agent.

Male Long Evans rats implanted with intracerebroventricular (icv) guide cannulae were subjected to one of the following treatments: (1) 2.5 µg SS icv on day 1 and 1.0 µg AVP icv on day 3; (2) 1.0 µg AVP icv on day 1 and 2.5 µg SS icv on day 3; and (3) 1.0 µg AVP icv on days 1 and 3. Behaviors were then recorded and scored according to a pre-determined behavioral code over a 10 minute period.

Rats receiving 1.0 µg AVP icv on days 1 and 3 exhibited an increase in convulsive behavior following the second administration of AVP. Rats receiving 2.5 µg SS icv on day 1 showed minimal changes in behavior, but when 1.0 µg AVP icv was administered 2 days later, all rats exhibited barrel rotations and myoclonic-myotonic convulsions. This type of behavior is normally seen following a second injection of AVP icv, therefore, SS had increased the sensitivity of the brain to the convulsive action of AVP. When the reverse protocol was performed, however, no such cross-sensitization was observed. Animals given 1.0 µg AVP icv on day 1 displayed periods of immobility and staring, as expected, but there was no convulsive behavior in response to 2.5 µg SS icv on day 3.

The present work demonstrates that SS sensitizes the brain to the seizure-inducing effects of AVP, but, under the present experimental conditions, AVP does not potentiate the response to SS. This may indicate that AVP's convulsive properties do not generalize in a similar manner or to the same degree as SS's.

This work was supported by the MRC of Canada. DMB is an MRC student; QJP is an MRC scholar.

224.9 NALOXONE EFFECTS ON SEIZURE SUPPRESSION AND KINDLING DEVELOPMENT. S. Caldecott-Hazard, L. Katznelson*, R.F. Ackermann, J. Engel, Jr. Dept. of Neurology, UCLA, Los Angeles, CA 90024.

Previous studies have found that endogenous opioid peptides are released by kindled seizures. These opioids have been hypothesized to play roles in postictal behavioral depression, seizure development, or in postictal seizure suppression (a stimulus, following a seizure by a short interval, elicits an afterdischarge (AD) with reduced or absent behavioral signs). In the present study, naloxone was used to simultaneously investigate the role of opioids in seizure development and postictal seizure suppression. Rats were implanted with bipolar stimulating electrodes in the amygdala and with recording electrodes on the cortex. Kindling was developed by stimulating at 60 Hz for 1 sec with a threshold sufficient to evoke an AD on the first stimulation. All rats were stimulated every 10 min for 1 hr per day, for 4 days. Naloxone (10 mg/kg,ip) was administered every 20 min to one-half of the rats and the control group received saline. The behavioral severity of the seizure ("kindling stage," Racine) and the AD duration were noted following each kindling stimulation. It was found that naloxone had no effect on the kindling rate as seen by the progressively increasing behavioral stages of the 1st stimulation on each day in both saline and naloxone rats. Also, the mean number of stimulation trials until each rat showed a fully generalized (stage 5) seizure was not significantly different between saline and naloxone rats. However, following the 1st stimulation of each day, the kindling stage for the saline rats decreased with each subsequent stimulation. In contrast, the naloxone rats maintained statistically significant higher kindling stages throughout the daily stimulations. Similarly, naloxone partially blocked the shortening of the AD durations seen following repeated seizures under saline conditions.

In order to study the effects of naloxone under conditions of increased postictal seizure suppression, rats were administered naloxone or saline and were stimulated every 10 min for 5 hrs on 1 day. The saline rats demonstrated moderate seizures (stages 1-2) for the 1st 2 hrs of stimulation, then the seizure stage decreased until ADs were often elicited without behavioral signs (stage 0). The naloxone rats maintained significantly more intense seizures (stages 2-4) for 3 hrs, followed by stage 1-2 seizures. Only one naloxone animal achieved a stage 5 seizure. AD durations were similar for all rats for the 1st 2 hrs. While the durations shortened in the naloxone rats, they first increased and then decreased in saline rats.

These data provide additional evidence that opioids may not be significantly involved in the development of kindling, but that they may play a role in a separate yet concurrent phenomenon, postictal seizure suppression.

224.10 EFFECT OF AMYGDALA KINDLING ON PYRIFORM CORTEX RESPONSE: INTRACELLULAR STUDIES WITH IN VITRO SLICE. D.C. McIntyre*, R.K.S. Wong and R. Miles (SPON: T. Tombaugh). Dept. Psychology, Carleton Univ. Ottawa, Ont. and Dept. of Physiol. and Biophysics, UTMB. Galveston, TX 77550.

The depletion of norepinephrine (NE) with neurotoxin 6-hydroxydopamine markedly facilitates the rate of amygdala kindling (e.g., McIntyre et al. Exp. Neurol.:1981). We now studied the cellular action of NE on neurons in the amygdala-pyriform area in order to assess the regulatory role of NE in the kindling-induced seizure activities. Experiments were carried out in vitro using coronal slices prepared from amygdala-kindled and implanted control Wistar rats. Antecedent amygdala kindling involved stimulating the right amygdala through a bipolar electrode with a 60 Hz sine wave of 50 or 100 μA twice daily until 6 generalized stage-5 convulsions were recorded. During intracellular studies, pyriform neurons were activated by stimulation of the basolateral, lateral or cortical amygdala nuclei via a bipolar tungsten electrode. In the control experiments two types of e.p.s.p.'s were evoked by such stimulation--first, an initial short latency event directly elicited by the shock and, second, a following barrage of recurrent e.p.s.p.'s generated via local neuronal interaction. In the kindled tissue, the recurrent e.p.s.p.'s were marked potentiated and were often sufficient to initiate synchronized population bursts as well as afterdischarges. NE (10^{-6}M) in the perfusate reversibly suppressed the recurrent e.p.s.p.'s in the control slices. Accordingly NE (10^{-6}M) also suppressed synchronized bursts and afterdischarges in the kindled tissue. Additional experiments showed that the α_2 agonist clonidine (10^{-6}M) produced the same effect as NE while the α_1 agonist phenylephrine (10^{-5}M) was ineffective. Interestingly the β agonist isoproteronol (10^{-6}M) appeared to potentiate the recurrent synaptic events.

The results indicate, as in vivo, that NE is an effective inhibitor of amygdala-pyriform neuronal activities and that this effect of NE is probably mediated via the presynaptic receptors.

224.11 AGE-RELATED CHANGES IN KINDLED SEIZURE DEVELOPMENT. K.A. Welsh and P.E. Gold. Department of Psychology, University of Virginia, Charlottesville, Virginia, 22901.

Considerable evidence in the Fischer F-344 rat indicates specific age-related declines on a variety of neurobiological and behavioral measures. The present study investigated possible age-related changes in the kindled seizure model of neuronal plasticity.

Bipolar stimulating electrodes were implanted bilaterally into the basolateral nucleus of the amygdala in both 3- and 12- month old Fischer F-344 rats. One week after surgery, afterdischarge (AD) thresholds were determined and kindling trials begun (unilateral, monophasic square waves, 60 Hz, 250 uA, 1 sec duration, one stimulation per day). Trials were continued for 30 days until each animal had demonstrated at least 3 consecutive stage 5 seizures. Stimulation was then discontinued and the permanence of kindling was tested two weeks later.

Prior to kindling, there were no significant differences in AD thresholds or durations in the 3- and 12- month old rats. In addition, the rate of kindling (as assessed by increases in AD duration) was comparable for the two age groups for at least the first 7 daily trials. Soon afterward, however, the AD durations digressed; 12-month old rats reached an asymptotic AD duration of 60 sec while the 3-month old rats continued to develop longer seizure durations over the next 3 weeks reaching an asymptote of 110 sec ($P < 0.01$). The development of behavioral convulsion stages (Racine 5 point scale) exhibited a pattern consistent with the electrographic results; aged rats required more trials (15.4 trials vs 10.7 trials) before demonstrating behavioral clonus. Despite these differing kindling rates, the kindled convulsions appeared to be permanent in each group; all animals exhibited convulsions on the first retest trial after a 2-week interval.

These findings indicate that the development of kindled seizures exhibits substantial age-related changes. Because AD thresholds and patterns are initially similar, the differences in kindling are probably not attributable to differences in sensitivity to the stimulation. Although the neurobiological basis of the age-related deficit in kindling remains to be clarified, these results are consistent with other physiological data suggesting impaired synaptic efficacy in the brains of aged animals (e.g. Landfield et al., Br. Res., 1978,150,85). Such functional changes may underlie the kindling deficit and prove important to the aging deficits observed in other forms of neuronal and behavioral plasticity.

Supported by NIA (AG 01642)and NIMH (MH 31141).

224.12 INTRACEREBRAL INFUSIONS OF COLCHICINE ABOLISH KINDLED EPILEPTOGENIC FOCI IN CATS. N.R. Carlson*, K.D. Laxer*, and M.A. Mason* (SPON: R. Dow). Good Samaritan Hosp., Portland, OR 97210. Previous studies have shown that kindled epileptogenic foci produce morphological and functional changes in neurons in parts of the brain remote from the stimulating electrode. To test the hypothesis that these changes are maintained by neural activity of cells in the vicinity of the focus we infused the region of the focus with colchicine. This drug temporarily interferes with various neural functions, including axoplasmic flow and generation of action potentials. Cannulas were made from 22-ga spinal hypodermic needles, insulated except for the tip and the stylet, which protruded approximately 0.5 mm. They were chronically implanted in the amygdalas of cats. Kindled seizures were produced by repeated daily electrical stimulation through the cannulas. After stage V or VI seizures were reliably produced and seizure stimulation thresholds were measured, the stylet was removed and colchicine (or control substances) were slowly infused (20 min) through 27-ga hypodermic needles inserted into the cannula. Brain stimulation resumed after a recovery interval of 13-15 days. Infusion of 15 uL of colchicine (2mg./ml.) abolished seizures even after stimulation intensity was increased to 1800-2500 uA (median preinfusion threshold, 500 uA) in five cats. In one cat seizures could be produced, but the threshold was raised from 400 uA to 1500 uA. In contrast, seizure thresholds were not elevated by control infusions of equivalent amounts of saline (two cats) or lumicolchicine (four cats). Infusions of 5 uL of colchicine (four cats) had small effects; data are being collected from cats receiving 10 uL. Histological examination showed that the effects were not related to tissue damage caused by the drug.

225.1 PRIMARY PROJECTIONS TO COCHLEAR NUCLEI FROM CHRONICALLY DEAF COCHLEAS. D.B. Webster. Kresge Laboratory, Department of Otorhinolaryngology, L.S.U. Medical Center, New Orleans, LA 70119.

Pigmented guinea pigs were deafened at one month of age by a subcutaneous injection of kanamycin (400 mg/kg) followed two hours later by an intravenous injection of ethacrynic acid (40 mg/kg). This drug treatment results in a loss of all cochlear hair cells (except about 20 outer hair cells in the apical turn). By eight months following drug treatment, the spiral ganglion neuronal population is reduced to 13% of its normal number; this residual population remains stable. In the present experiment, the guinea pigs were allowed to survive for 18 to 29 months following drug treatment. Ten were then given discrete lesions of the spiral ganglion in the basal, second, or apical turn, allowed to survive for 4 additional days, and then anesthetized and perfused with 10% formalin. Their cochleae were studied to determine the extent of lesioning; their brainstems were examined by the Fink-Heimer method for axonal degeneration. Another 10 animals were treated with horseradish peroxidase (Sigma VI, 30%) placed on the cochlear nerve which had been surgically severed at the apex, second turn, or basal turn. These guinea pigs were sacrificed 24 to 48 hours later and their brainstems studied using the tetramethyl benzidine method. The results demonstrate that the residual spiral ganglion neurons of chronically drug-deafened guinea pigs project to the cochlear nuclei in a cochleotopic pattern which is indistinguishable from that seen in normal guinea pigs, save that it is much sparser.

Supported by NIH grants NS-12510 and NS-11647.

225.2 TUNING PROPERTIES OF SINGLE UNITS IN THE VENTRAL COCHLEAR NUCLEUS OF GUINEA PIGS IN RESPONSE TO ELECTRICAL STIMULATION OF THE COCHLEA. I. Glass* (SPON: M.J. Mustari). Dept. of Physiol. and Biophys., Univ. of Washington, Seattle, WA 98195

Tuning properites of auditory neruons to electrical stimulation of cochlear afferents are relevant to selection of stimulation frequencies and electrode locations for auditory neural prostheses. Single units in the ventral cochlear nucleus of acutely anesthetized guinea pigs were tested with pure continuous sinusoids presented through a tubular free fit implant in the scala tympani. Implants had two or four electrodes along the axis of the scala with 1 mm separations.

Best frequencies were consistently in the 100 Hz range (50-250 Hz) with thresholds of about 0.063 mA p-p. Tuning curves were usually symmetrical with slopes of 3-4 dB/octave, both below and above the best frequency. The relative sharpness of the tuning curves, as measured by Q_{10}, averaged 0.2. Dynamic ranges as determined by saturation of the rate-intensity functions for the various frequencies were 3-15 dB. No significant difference was found in these measures between responses evoked by the apical vs. the basal pairs of electrodes.

Units were found to have phase-locked responses to electrical stimulation of frequencies up to 12800 Hz. When tested with two pairs of electrodes, the frequency following patterns of a given unit to each pair were usually distinct.

These observations suggest that the electrical signal stimulates selective parts of a cochlear nerve fiber (e.g., dendrites vs. axons), and/or different populations of cochlear nerve fibers innervating the same cochlear nucleus neuron.

225.3 PERSISTENCE OF CALYCES OF HELD IN THE MEDIAL NUCLEUS OF THE TRAPEZOID BODY AFTER PERINATAL TRANSNEURONAL DEGENERATION OF THE CONTRALATERAL VENTRAL COCHLEAR NUCLEUS. W.B. Warr, K.W. Nordeen* and L.M. Kitzes*. Boys Town National Institute for Communication Disorders in Children, Omaha, NE 68131, and Department of Anatomy, College of Medicine, University of California, Irvine, CA 92717.

It is well established from studies in the cat and rat that the globular cells of the ventral cochlear nucleus (VCN) project via large diameter axons to the medial nucleus of the trapezoid body (MNTB) of the opposite side. Moreover, these globular cells are the source of the large cup-shaped terminals, the calyces of Held, found one per cell on the principal cells of MNTB. In the present study, the right and left MNTBs were examined in Bodian-stained material from adult gerbils subjected at 2 days of age to destruction of one cochlea. This ablation invariably resulted in the complete transneuronal degeneration of the VCN. However, despite the degeneration of the entire large-fiber component in the ipsilateral ventral acoustic stria, the MNTB contralateral to the ablation contained many large-diameter axons and many principal cells with a calyx terminal. The MNTB ipsilateral to the ablation appeared similar to that of unoperated controls. These findings indicate that the neonatal cochlear ablation and the resulting transneuronal degeneration of VCN produces a remarkable alteration of axonal connections within the adult auditory brain stem. The development or maintenance of this anomalous innervation of the MNTB may provide an indication of the normal process of development in this nucleus. (Supported by NSF Grant BNS-82-09987 to W.B. Warr, and NIH Grant NS-17596 to L.M. Kitzes.)

225.4 FINE STRUCTURE OF THE LATERAL SUPERIOR OLIVARY NUCLEUS IN THE ALBINO RAT. J.S. White. Dept. of Anatomy, Creighton Univ. Sch. of Med., Omaha, NE 68178.

The lateral superior olivary nucleus (LSO) is one of the three main auditory nuclei of the superior olivary complex involved in the processing of binaural input. The major cell type in this nucleus, the principal cell, is known to receive input directly from the ipsilateral anteroventral cochlear nucleus and from the contralateral anteroventral cochlear nucleus by way of an intervening synapse in the medial nucleus of the trapezoid body. A recent study of the origins of the olivocochlear bundle in the rat indicates that the LSO also contains a population of small olivocochlear neurons that project exclusively to the ipsilateral cochlea. The present study was undertaken to examine the morphology of, and the types and arrangement of synaptic terminals on each of these two types of cells.

In thin-sectioned material, principal cells typically display large central nuclei with a slightly infolded nuclear envelope, and abundant cytoplasm. In many instances, a large dendrite could be seen emanating from one or both poles of the cell body, and extending for some distance into the surrounding neuropil. Almost the entire surface of the cell soma and the proximal parts of the dendrites are contacted by numerous synaptic terminals containing either rounded or flattened vesicles. Terminals with flattened vesicles were found predominantly on the cell soma, while those with rounded vesicles usually were apposed to the dendrites. Olivocochlear neurons in the LSO are smaller, and display nuclei with highly infolded nuclear membranes. The somatic surface of these cells are contacted by only a few synaptic terminals that contain rounded vesicles.

It seems reasonable to suggest that the two types of terminals in synapse with the principal cells are related to the two known sources of input to this nucleus. It remains to be determined, however, if either the anteroventral cochlear nucleus or the medial nucleus of the trapezoid body also project to the small olivocochlear neurons in the LSO. (Supported by a grant from the Deafness Research Foundation).

225.5 THE FINE STRUCTURE OF THE LATERAL SUPERIOR OLIVARY NUCLEUS OF THE CAT. Nell Beatty Cant. Department of Anatomy, Duke University Medical Center, Durham, NC 27710.

The synaptic organization of the middle limb of the lateral superior olivary nucleus of the cat was analyzed in the electron microscope. The predominant cell type--the fusiform cell--has dendrites that extend from opposite poles of the cell body toward the margins of the nucleus, where they terminate in distal, spiny branches. Approximately 85% of the somatic and proximal dendritic surfaces are apposed by large synaptic terminals containing small, flat synaptic vesicles. Away from the cell body, the dendrites form numerous synaptic contacts with large synaptic terminals containing large, round vesicles in addition to contacts with the terminals with flat vesicles. The most distal dendritic branches and their spiny appendages appear to form synapses almost exclusively with the terminals with large, round vesicles. A very rare type of terminal that contains small, round vesicles may form synapses with either the somatic or dendritic surfaces. A few small cells are interspersed among the fusiform cells, but they are more commonly located around the margins of the nucleus. The small cells form few axosomatic contacts.

It is likely that the terminals with small, flat vesicles arise in the medial nucleus of the trapezoid body and are inhibitory in function, whereas the terminals with large, round vesicles arise in the anteroventral cochlear nucleus and are excitatory. The differential distribution of the two inputs on the somatic and dendritic surfaces must be an important determinant of the physiological response properties of these cells to binaural acoustic stimuli.

Supported by NIH grant NS14655.

225.6 LAMINAR PROJECTIONS TO THE INFERIOR COLLICULUS AS SEEN FROM INJECTIONS OF WHEAT GERM AGGLUTININ-HORSERADISH PEROXIDASE IN THE SUPERIOR OLIVARY COMPLEX OF THE CAT. J.H. Casseday and E. Covey, Div. of Otolaryngology, Duke Univ. Med. Center, Durham, N.C. 27710.

Small injections of wheat germ agglutinin conjugated to horseradish peroxidase were placed in divisions of the superior olivary complex via microelectrodes that were also used to monitor the electrophysiological response to tone bursts. Injections in lateral superior olive (LSO) and medial superior olive (MSO) resulted in bands of anterograde label in the lateral and central parts of the central nucleus of the inferior colliculus. In frontal sections, these labeled areas appear as vertical bands tilted slightly from ventrolateral to dorsomedial. After very small injections only a single band is seen, approximately 100 μm wide, but it often extends throughout most of the central nucleus in the caudal to rostral and dorsal to ventral dimensions. After larger injections several bands are seen. Between each band is a label-free area approximately equal in width to the band itself. Injections in low-frequency areas of LSO produce bands in lateral parts of the inferior colliculus. The same area of the inferior colliculus is labeled after injections in the low frequency area of MSO but it is not clear if the bands from LSO are superimposed on the bands from MSO. Injections in high-frequency areas of LSO produce bands in medial locations. The projection bands are always located in the inferior colliculus ipsilateral to the injection and appear bilateral only after injections in LSO. That these bands of label are not merely projections to areas rich in cells or sparse in cells is shown by the observation that cells are seen in equal proportion both within and adjacent to the bands. An injection in the dorsomedial periolivary area yielded widespread, diffuse anterograde labeling throughout most of the inferior colliculus, including both central and pericentral areas, suggesting that not all ascending projections to the inferior colliculus have a laminar organization. Retrograde transport after small injections in LSO revealed bands of labeled cells in the anterior divisions of the anteroventral cochlear nucleus. These bands were oriented in a dorsolateral to ventromedial direction, probably in register with isofrequency contours. The results raise the possibility that the laminar projections from LSO and MSO interleave or overlap with projections from some other source or with one another. [Supported by NIH grant NS 12322.]

225.7 Comparison of responses to interaurally delayed low frequency tonal and wide-band noise stimuli in the cat's inferior colliculus. Joseph C.K. Chan, Dexter R.F. Irvine, Alan D. Musicant*, and Tom C.T. Yin. Dept. of Neurophysiology, Univ. of Wisconsin, Madison, WI. 53706.

Many low frequency neurons in the central nucleus of the inferior colliculus (ICC) of the cat are sensitive to variations in interaural time differences (ITD's). This sensitivity is manifested in cyclic interaural delay curves at different frequencies within the neuron's response area. Most studies of delay sensitivity have used pure tones, and there is little information on delay sensitivity to more natural, spectrally-complex sounds. We have examined the delay sensitivity of ICC neurons using band-pass filtered noise and have tested the hypothesis that the response to noise can be predicted from the responses to pure tones.

Experiments were carried out on adult cats anesthetized with sodium pentobarbital. Conventional dichotic stimulation and extracellular recording techniques were used. Low-pass Gaussian noise sequences with a flat spectrum up to a specified cut-off frequency (usually 4 kHz) were generated digitally. The tone and noise stimuli were delivered from a digital stimulus system. The binaural beat stimulus was used to obtain data on pure tone delay sensitivity over the entire response area of the cell.

The results indicate that cells with periodic ITD sensitivity functions for tonal stimuli also show delay sensitivity to noise. The noise curves are characterized by either a maximum or a minimum response within the physiological range of ITD for the cat (about 350 usec). For most cells, the shape of the noise delay curves is invariant with stimulus intensity. The extent to which a given cell's response to noise can be predicted from its response to tones has been assessed by comparing the noise curve with a composite tone curve, obtained by averaging the delay curves for individual frequencies. For most neurons, there is a close correspondence between the noise and composite tone delay curves: the general shapes of the two curves are similar and the maxima, minima and slopes occur at approximately the same ITD's. Thus, it appears that the delay sensitivity of ICC cells to low-pass noise can be predicted from their responses to tones.

When narrow-band noise signals are used, the periodicity of the delay curve depends upon the spectral composition of the signal. Studies are in progress to determine whether the delay curve for narrow-band noise restricted to a portion of the cell's response area matches the composite tone curve averaged over the corresponding frequency range.

Supported by N.I.H. grants NS12732, EY02606 and NS07026.

225.8 EFFECTS OF TRANSECTING THE THREE ACOUSTIC STRIAE ON THE AUDITORY UNIT ACTIVITIES OF THE INFERIOR COLLICULUS IN THE CAT, Paul W. Y. Hui* (SPON: William D. Neff). Center for Neural Sciences, Indiana University, Bloomington, IN 47401

Auditory units were isolated in the inferior colliculus (IC) before and after transections of the trapezoid body (TB), the intermdeiate acoustic stria (IAS) and the dorsal acoustic stria (DAS). Units isolated on the same tracks before and after the transecions were compared. It was found that:

1. Partial lesion of the TB reduced the population of the binaural units and the contralateral excited units. The ipsilateral unit population was proportionally increased. The values and ranges of the latencies after the transections were also increased. The post-stimulus histogram (PSH) changed from the conherent type to the more diffuse type.

2. Complete transection of the TB did not eliminate all the binaural units nor the contralaterally excited monaural units.

3. Complete transections of the TB, IAS and DAS eliminated all the contralaterally excited monaural units. A small population of binaural units was still remained. This indicated that the IC also processed binaural inputs from neural centers other than the superior olivary complex (SOC).

4. The percentage increase of ipsilaterally excited monaural units in all the cases was the same as the percentage decrease of binaural units after the lesions.

225.9 A MAP OF AUDITORY AZIMUTH AND ELEVATION IN THE CAT'S SUPERIOR COLLICULUS. J.C. Middlebrooks and E.I. Knudsen. Dept. of Neurobiology, Stanford University, Stanford, CA 94305.

We have explored the representation of sound space in the cat's superior colliculus (SC) by measuring the selectivity of neurons for sound location and correlating this spatial tuning with the location of neurons in the SC. Single units were recorded extracellularly in anesthetized adult cats. Auditory stimuli consisted of noise bursts generated by a movable speaker. Visual stimuli were projected on a hemisphere which was placed in front of the cat during visual testing. Recording sites were marked with electrolytic lesions and later were identified histologically.

Unit responses to acoustic stimulation were encountered in the intermediate and deep layers. All auditory units were selective for sound location. Although some units had delimitable auditory receptive fields, others exhibited above background responses to stimuli presented at any location. However, regardless of the size of its receptive field, each unit exhibited spatial tuning in the form of a peak response to a limited range of stimulus locations. We compiled spatial response profiles of single units by counting the number of spikes elicited per stimulus presentation as the location of the noise source was varied systematically in azimuth and elevation. The best area of a neuron was defined as the area in which sound stimuli elicited responses greater than 75% of maximum. The centers of best areas ranged in azimuth from ipsilateral 30^{o} to contralateral 100^{o} and in elevation from $+45^{o}$ to -60^{o}. Many auditory units also responded to visual stimulation. For each bimodal unit, the centers of its visual field and auditory best area were approximately aligned.

The best areas of units varied systematically with their locations within the SC. Best areas shifted in azimuth from frontal to contralateral along a rostral to caudal axis and in elevation from superior to inferior along a medial to lateral axis. In the intermediate gray, azimuths from 0^{o} to 80^{o} contralateral were mapped continuously with a slight relative magnification of frontal space. Most of the mediolateral axis in that layer was devoted to representing a narrow range of 35^{o} in elevation located around the visual plane. The representation of higher and lower elevations was markedly compressed. Although some units were tuned sharply for locations outside of the visual range, the range and internal topography of the auditory map conformed closely with that of the visual map. In addition to revealing the topography of the map of auditory space in the SC, these data suggest the form in which space might be represented elsewhere in the central auditory system.

March of Dimes Foundation grant 5-285; NIH grants R01 NS16099 03 and 5 F32 NS06992-02

225.10 EARLY AUDITORY EXPERIENCE ALIGNS THE AUDITORY MAP OF SPACE IN THE OPTIC TECTUM OF THE BARN OWL. E. I. Knudsen. Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

The optic tectum contains maps of auditory and visual space that are mutually aligned. At the single unit level, this alignment is manifested as a coincidence of the optimal locations of auditory and visual stimuli. How does the alignment of spatial maps from different sensory modalities come about? Is the underlying circuitry shaped by experience? To investigate this question I altered in young barn owls the binaural localization cues associated with each point in space, then examined the tectum for changes in the auditory map. Binaural cues were disrupted in four young barn owls (ages 31 to 45 days) by suturing a foam rubber plug into one external meatus. The experimental birds and 3 control birds were raised to maturity (7 months) in an aviary. As adults, these birds were fitted with chambers for repeated neurophysiological recording from the tecta. Each owl was anesthetized with ketamine hydrochloride and positioned in a sound room containing a speaker-movement system. Noise bursts were used to determine auditory receptive fields and best areas (location for maximal response); moving bars and spots projected onto a hemisphere were used to map visual receptive fields. The hemisphere was removed during auditory testing.

In control birds, auditory best areas and visual receptive fields of units in the rostral portion of the tectum exhibited a mean misalignment of 0.0° in azimuth and -2.1° in elevation (n = 46). In three experimental birds with ear plugs still in place, the average misalignments were respectively 0.0° azimuth, -2.3° elevation; right 1.8°, +4.8°; and right 4.5°, +3.6°. The fourth owl was not recorded from before its ear plug was removed. The approximate alignment of fields in birds experiencing altered binaural inputs indicates that these bimodal units had developed new specificities for binaural cues spatially consistent with their visual fields.

When the ear plugs were removed, the auditory fields and best areas moved abruptly away from the side of the unplugged ear causing the auditory and visual fields to become grossly misaligned. The misalignment of the auditory and visual receptive fields persisted for up to 5 months.

The results demonstrate that the alignment of the auditory map of space in the optic tectum is regulated by early auditory experience. The persistence of the altered auditory map in adult birds suggests that the mechanism for adjusting neuronal specificities for binaural cues is lost or greatly slowed in the adult nervous system.

This work was supported by March of Dimes grant # NF 5-285 and NIH grant # NS 16099-03.

225.11 AURAL REPRESENTATION WITHIN AN ISOFREQUENCY LAMINA OF THE MUSTACHE BAT'S INFERIOR COLLICULUS. L.S. Ross*, J.J. Wenstrup*, and G.D. Pollak. (SPON: R. Bodenhamer). Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

The greater mustache bat, Pteronotus parnellii, uses a constant frequency echolocation call in which most of the energy is concentrated in the 60 kHz second harmonic. Within the central nucleus of the inferior colliculus, there is a large architectonically defined subdivision, the dorsal posterior division (DPD), occupying almost 35% of its volume. The DPD is an isofrequency lamina in which all neurons have the same best frequency that corresponds to the 60 kHz component of the echolocation calls. In this study, we analyzed the aural representation of neurons in the DPD and how these features are mapped upon this subdivision.

Three types of units were observed; 1) E-O units were excited by contralateral stimulation but were unaffected by ipsilateral stimulation; 2) E-E units were excited by both contralateral and ipsilateral stimulation; and 3) E-I units whose excitatory responses to contralateral stimulation could be inhibited by ipsilateral stimulation.

Each type was segregated within the DPD and occupied regional territories: E-O units were found dorsally and laterally: E-E units were found in bands located ventro-laterally; while E-I units occupied a thick band in the medial portion of the DPD, sandwiched between the E-O band dorsally and the E-E band ventrally. Thus within this isofrequency lamina, units having similar binaural properties are topographically arranged. It seems likely that this arrangement is a consequence of the distribution of converging inputs from lower acoustic centers. Supported by grants from the NIH and NSF.

225.12 TOPOGRAPHICAL REPRESENTATION OF INTERAURAL INTENSITY DIFFERENCES WITHIN AN ISOFREQUENCY LAMINA OF THE MUSTACHE BAT INFERIOR COLLICULUS. J.J. Wenstrup*, L.S. Ross*, and G.D. Pollak. Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

Recent experiments have demonstrated that different classes of binaural response properties are segregated in isofrequency contours of mammalian auditory centers, yet the neural organization of responses within each of these classes has remained poorly understood. We now report the results of experiments in which we find systematic shifts in E-I response properties with depth in a single isofrequency lamina of the inferior colliculus of the mustache bat, Pteronotus parnellii.

Responses of single units and unit clusters to monaural and dichotic sound stimuli were obtained within the dorsal posterior division (DPD) of the mustache bat inferior colliculus. Neurons within the physiologically and anatomically distinct DPD are tuned to the 60 kHz constant frequency component of the bat's sonar pulse.

E-I responses were found in the medial region of the DPD, ventral to a band of E-O units. Within this region, dorsoventral electrode penetrations first encountered E-I responses in which the ipsilateral stimulus inhibited the excitatory response to contralateral sound at intensities typically 30 dB greater than the contralateral stimulus. With increasing electrode depth, inhibition by the ipsilateral sound occurred at successively lower intensities relative to the contralateral stimulus. The deepest E-I units encountered in a penetration were inhibited by ipsilateral sound intensites 0 to 10 dB below the contralateral sound. This pattern of E-I responses was clearly observed in most of the medial penetrations through the E-I band. Cases in which successive E-I units did not display a systematic shift in ipsilateral inhibitory thresholds were generally located more laterally.

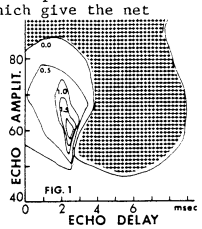
Such organization is likely to provide a neural substrate of sound localization dependent upon intensity cues, and may thus constitute an internal representation of external auditory space.

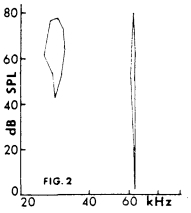
Supported by grants from the NIH and NSF.

225.13 COMBINATION-SENSITIVE NEURONS IN THE AUDITORY THALAMUS OF THE MUSTACHED BAT. J.F. Olsen* and N. Suga. Biology Dept., Washington U., St. Louis, MO 63130

We have been recording extracellularly from single units in the auditory thalamus of the mustached bat (Pteronotus parnellii). The biosonar signal (pulse) of this bat consists of a long constant-frequency component (CF) which terminates with a brief frequency-modulated component (FM). The auditory cortex contains two types of neurons (FM-FM and CF/CF) which are sensitive to combinations of signal elements in the pulse-echo pair (Suga et al., Science 203:270, 1979; Suga and O'Neill, Science 206:351, 1979). The responses of CF/CF and FM-FM neurons are respectively facilitated by the CF or FM component of the fundamental of the pulse plus the CF or FM compononent of a higher harmonic of the echo. CF/CF neurons are tuned to Doppler-shifted echoes, whereas the response of FM-FM neurons encodes echo delay. We have found similar CF/CF and FM-FM neurons within the auditory thalamus. Most thalamic FM-FM neurons are tuned to particular echo delays between 0.4 and 18 msec. Figure 1 illustrates the response of an FM_1-FM_2 neuron as echo delay and amplitude are varied. This unit responds weakly to the echo FM_2 presented alone, a response which is facilitated by the addition of the FM_1 of the pulse. The lines are isoimpulse-count contours which give the net increase in response evoked by the stimulus pair. The trailing edge of this neuron's delay tuning curve is sharpened by the presence of an adjacent inhibitory area (shaded in figure). Figure 2 shows a facilitative tuning curve for a thalamic CF_1/CF_2 neuron. Typically these tuning curves are not sharply tuned to the fundamental, but very sharply tuned to the second harmonic. Figures 1 and 2 also illustrate that FM-FM and CF/CF neurons are tuned to particular echo amplitudes. We have also found neurons which are tuned to single CF or FM stimuli. Since combination-sensitivity has not yet been discovered in the ascending auditory pathway below the thalamus, it seems likely that this response property may originate within the thalamus itself.

(Supported by PHS1-R01-NS17333-01).





REGENERATION: CENTRAL III

226.1 MATURE OLIGODENDROCYTES HAVE THE CAPACITY TO DIVIDE FOLLOWING EXPERIMENTAL DEMYELINATION IN THE ADULT CENTRAL NERVOUS SYSTEM. Lynn S. Arenella* and Robert M. Herndon. Center for Brain Research, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

While neurons are generally considered to be non-dividing cells, the mitotic capacity of glial cells is an issue which has been debated since the turn of the century. Today it is accepted that astrocytes and microglia have the capacity to divide in response to injury, disease, and in the normal course of aging, but a similar capacity for mature oligodendrocytes remains a controversial issue. There is, however, substantial evidence that replacement of oligodendrocytes occurs following demyelination and preceding remyelination both in vivo and in vitro. The origin of these oligodendrocytes remains unclear. They could arise from the division of pre-existing oligodendrocytes or from the division and subsequent differentiation of unspecialized precursor cells. In order to study this problem, we combined autoradiographic techniques, utilizing a pulse label of ^{3}H-thymidine to determine the identity of cells about to undergo mitosis, with the lysolecithin-induced model of primary demyelination.

Swiss mice, 9 to 10 months of age, were each injected with 2 µl of 1% lysolecithin in saline in the thoracic region. Mice were injected i.p. with 5.0 or 14.0 µCi/gm (methyl-^{3}H)thymidine and perfused 1 hr later on days 2, 5, 8 and 28 post-lysolecithin. Serial thick (1.5 µm) and thin sections were prepared for light and electron microscopic autoradiography.

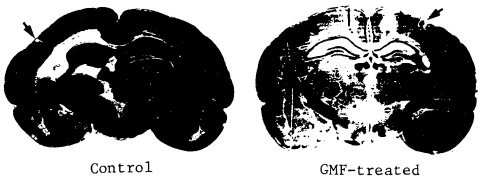
Two days after lysolecithin injection, labeled macrophages were present both within the immediate vicinity of the injection site and in the surrounding area where by day 5 they had removed enough myelin debris to produce a discrete edematous zone of bare axons. Just outside this area of myelin destruction, labeled astrocytes, first evident at day 2, were numerous at days 5 and 8. Labeled oligodendrocytes, although rare at day 2, appeared first outside the zone of myelin destruction. At days 5 and 8, numerous labeled oligodendrocytes could be found in the zone surrounding the injection site where remyelination, barely apparent at day 5, was well underway at day 8. By day 28, all surviving axons had been remyelinated, many, in fact, by invading Schwann cells.

The presence of dividing oligodendrocytes before myelin debris has been cleared from most axons suggests that oligodendrocytes, like astrocytes, have the capacity to divide in response to injury. While the presence of bare axons may further influence the proliferation and migration of these cells, it does not seem to be an essential requirement for the regeneration of oligodendrocytes in these lesions. The finding of oligodendrocytes capable of dividing in older animals, lends support to observations of an active multiple sclerosis lesion suggesting "an apparent proliferation" of these cells in the plaque margin (Raine et al., 1981). Our results show that oligodendrocyte proliferation precedes and accompanies successful regeneration of CNS myelin.

226.2 IN VIVO EFFECT OF GLIA MATURATION FACTOR ON CEREBRAL REGENERATION. Ramon Lim and Joyce F. Miller*. Div. of Neurochemistry and Neurobiology, Dept. of Neurology, Univ. of Iowa, Iowa City, IA

Glia Maturation Factor (GMF), first detected in our laboratory in 1972, is a 15,000 dalton acidic protein capable of stimulating astrocytic proliferation and differentiation in vitro. We now report the in vivo effect of GMF in enhancing cerebral regeneration. Newborn rats were injured in the parietal region on one side of the brain by piercing an 18 gauge needle through the skull, creating a puncture wound 4 mm deep. Five litters of rats were used for the study and rats from each litter were randomly assigned into experimental and control groups. Experimental animals (N=26) were treated with intraperitoneal injections of beef GMF (50 nanograms GMF in 10 ul of 0.2 M phosphate buffer, pH 7.4) on 3 consecutive days starting from the time of injury, whereas control animals (N=24) were treated with similar injections where GMF was replaced by 50 nanograms of bovine serum alumin. Twenty-five days after birth, the brains were removed, fixed, paraffin-embedded, and serial coronal sections 10 microns thick were made and stained with H & E. One representative section from each brain, cutting through the region of maximal injury, was photographed and the areas defined by the left and the right cerebral hemispheres were measured with a digitized planimeter.

The measurements demonstrated that in control animals the cerebral hemisphere of the injured side was 19% smaller than the normal side ($p < .0001$), but in the GMF-treated animals no difference in size was detected. Analysis of variance showed that the effect of GMF was highly significant ($p < .0001$) and could not be attributed to sex or litter differences. No evidence of glial scar formation was seen at the site of injury upon immunostaining for Glial Fibrillary Acidic Protein, whether or not the rats were treated with GMF. The results indicate that GMF promotes cerebral regeneration and suggest a potential therapeutic value for GMF in brain damage. (Supported by NIH grant CA-31796 and NSF grant BNS-8308341 and by a Veterans Administration Career Investigatorship to R.L.)



226.3 NORMAL DEVELOPMENT AND REGENERATIVE GROWTH OF NORADRENERGIC FIBERS TO THE LATERAL GENICULATE NUCLEUS OF THE RAT. L.L. Rose* and P.W. Land. Department of Anatomy, M.U.S.C., Charleston, SC 29425 and University of Pittsburgh, Pittsburgh, PA 15261.

Locus coeruleus axons have the capacity to reinnervate certain regions of the brainstem, but not cortex, following postnatal lesions with the neurotoxin 6OHDA. This ability may be correlated both with the proximity of the innervated region to the locus coeruleus and the maturational stage of the normal noradrenergic (NA) innervation of the region at the time of neurotoxic lesion. We studied the normal development of the NA innervation of the dorsal lateral geniculate nucleus (dLGN) of the pigmented rat and the effect of early postnatal 6OHDA treatment. Litters of Long-Evans rats were divided into two groups: control (uninjected) and 6OHDA-treated. We administered 6OHDA (Regis; 100 mg/kg free base in 0.5% ascorbate) on postnatal days 0, 1, 2 and 3. Control and 6OHDA-treated animals were sacrificed on days 0 through 10, day 21, and as adults. The younger animals (P0-P10) were perfused with a glyoxylic acid solution (Lidov, et.al., 1978) and reacted with the SPG method of De La Torre (1980). For the older ages, fresh tissue was reacted according to this procedure.

The adult dLGN receives an extremely dense, intensely fluorescent NA innervation which sets it apart from surrounding thalamic nuclei. The innervation develops postnatally, with only a few delicate fluorescent terminals present on P0. The number and intensity of fluorescent fibers increases daily, and by P10 the pattern resembles that of the adult, although it is less dense.

A single injection of 6OHDA was sufficient to cause the disappearance of almost all fluorescent terminals in the dLGN. Continued treatment until P3 maintained this depletion, and few NA fibers were visualized in the dLGN until the second postnatal week. By day 8, the innervation resembled that of the control P0 animal. The number of fibers continued to increase, and by day 21 the innervation was quite heavy but still not as dense as in controls. No cortical reinnervation by NA fibers was seen during this time.

These results show that, unlike the cortical NA projection, NA axons are able to reinnervate the dLGN after postnatal neurotoxic lesion. In addition, NA fibers normally innervate the dLGN after retinal afferents have reached the geniculate, but before they exhibit adult organization. This allows for a possible influence of noradrenergic fibers on the final pattern of afferent termination in the geniculate. (Supported by NIH grant EYO3447).

226.4 EFFECT OF GM_1 GANGLIOSIDE TREATMENT ON THE RECOVERY OF DOPAMINERGIC NIGRO-STRIATAL NEURONS AFTER DIFFERENT TYPES OF LESION. G. Toffano, G. Savoini*, C. Aldinio*, M. Valenti*, K. Fuxe* and L.F. Agnati*. Department of Biochemistry, Fidia Research Laboratories, 35031 Abano Terme, Italy, Department of Histology, Karolinska Institutet, 104-01 Stockholm, Sweden, Department of Human Physiology, University of Modena, 41100 Modena, Italy.

Many types of central neurons exhibit capacity for axon sprouting and generation of new connections in response to injury, not only in the developing but also in the mature CNS (Björklund A. and Stenevi U., Physiol. Rev., 59:62-100, 1977; Cotman C.W. et al., Physiol. Rev., 61:684-784, 1981). The intensity of CNS tissue to recover after injury seems to depend on the extent and localization of the damage, and on the presence of factors with repressive or stimulatory neuronotrophic activity. Among the factors affecting neurite growth response, a particular role has been recently attributed to GM_1 monosialoganglioside. Indeed GM_1 potentiates the NGF-induced differentiation of pheochromocytoma PC12 and DRG dissociated cells in culture, conversely antibodies against gangliosides prevent the neurite-promoting effect of NGF in DRG and the neuronal outgrowth from regenerating goldfish retinal explants. We have recently demonstrated that GM_1 administration facilitates the dopaminergic reinnervation of the striatum (Toffano G. et al., Brain Res., 261:163-166, 1983) and apparently maintains the number of dopaminergic cell bodies in the substantia nigra (Agnati L.F. et al., this meeting) after unilateral hemitransection in adult rats. However, GM_1 fails to stimulate the recovery of nigro-striatal dopaminergic markers when dopaminergic neurons are lesioned by injecting directly 6OH-DA (from 2 to 8 g) in the MFB (A 3.5, L 2.0, V 7.3; König Klippel Atlas). The different process involved in the degeneration of dopaminergic neurons after the mechanical or the chemical lesion may be relevant for understanding the mechanism of action of GM_1 on neuronal repair in vivo after injury. Moreover, when 6OH-DA (4 g) is injected in the ispilateral or contralateral MFB of animals with unilateral hemitransection and treated for 15 days with 30 mg/kg GM_1, the ganglioside effect is abolished.

These results indicate (i) that the recovery of biochemical and immunohistochemical parameters of the nigro-striatal dopaminergic pathways induced by GM_1 treatment occurs only in a certain type of lesion; (ii) that the terminal sprouting of DA fibers in the striatum after hemitransection is a response of the ipsilateral unlesioned axons and (iii) that the contralateral nigro-striatal pathway may play a role in the regulation of such a response.

226.5 CHRONIC GM-1 TREATMENT CAN PROTECT AGAINST RETROGRADE CELL BODY DEGENERATION IN THE ASCENDING DOPAMINE PATHWAYS AND INDUCE MORPHOFUNCTIONAL RECOVERY IN THE STRIATAL DOPAMINE NERVE TERMINAL SYSTEMS FOLLOWING PARTIAL LESIONS. L.F. Agnati, K. Fuxe*, L. Calza*, C. Farabegoli*, L. Cavacchioli*, F. Mascagni*, G. Toffano* and M. Kalia*. (SPON: J.L. Osterholm). Dept. of Human Physiology, University of Modena, Modena, Italy.

Male Sprague-Dawley rats have been hemitransected at the level of the mesodiencephalic junction producing a partial lesion of the mesostriatal dopamine (DA) pathway. Chronic treatment with the ganglioside GM-1 was initiated immediately following the operation and lasted for 56 days (10 mg/kg, i.p., daily). The tyrosine hydroxylase immunocytochemistry, using both the indirect immunofluorescence method and the PAP method, and amine fluorescence histochemistry were performed to demonstrate the various types of DA cell bodies, dendrites and nerve terminal systems. The amine turnover was analyzed by measuring the catecholamine fluorescence at various time intervals following treatment with the tyrosine hydroxylase inhibitor α-methyl tyrosine methyl ester. The DA receptors were analyzed by means of quantitative receptor autoradiography using the radioligands ^{3}H-spiperone and ^{3}H-N-propylnorapomorphine. Chronic GM-1 treatment induced on the lesioned side a blockade of the retrograde cell body degeneration, increased the density and length of DA dendrites and produced a regeneration of the DA innervation of the striatum by inducing regrowth from surviving DA nerve terminal systems (collateral sprouting). At the postsynaptic level GM-1 treatment prevented the appearance of DA receptor supersensitivity as well as the shrinkage of the striatal area seen on the lesioned side without ganglioside treatment. These changes in the characteristics of the DA receptors were associated with the prevention of the development of the behavioural signs of DA receptor supersensitivity as revealed in studies on rotational behaviour using apomorphine. In intact animals chronic treatment with GM-1 also produced effects on the ascending DA systems by reducing DA turnover within the large diffuse (CCK-negative) DA nerve terminal networks of the nuc. accumbens and the nuc. caudatus putamen. These results underlined that gangliosides not only can induce "trophic" actions, but also effects on the transmission processes in the intact DA neurons. Gangliosides may represent a new type of Parkinson's disease making possible not only symptomatic treatment as with l-dopa, but a true cure by counteracting the degenerative processes within the DA neurons of the substantia nigra.

226.6 EFFECT OF GM_1 GANGLIOSIDE ON MONOAMINE NEUROTOXIN INDUCED ALTERATION OF THE POSTNATAL DEVELOPMENT OF NORADRENALINE AND SEROTONIN NEURONS. G. Jonsson*, H. Kojima* and A. Gorio (SPON: H. Aldskogius). Dept. of Histology, Karolinska Institutet, Stockholm, Sweden and Fidia Research Lab., Abano Terme, Italy.

Exogenous administration of gangliosides has been shown to promote neuronal regrowth processes and functional recovery after a nerve damage. It was therefore considered of interest to investigate the effect of GM_1 ganglioside treatment on the alteration of the postnatal development of noradrenaline (NA) and serotonin (5-HT) neurons in rat brain produced by the selective monoamine neurotoxins 6-hydroxydopamine (6-OH-DA; a catecholamine neurotoxin) and 5,7-dihydroxytryptamine (5,7-HT; a 5-HT neurotoxin) respectively. The effects were analyzed using neurochemical (measuring endogenous monoamine levels and ^{3}H-monoamine uptake) and histochemical (Falck-Hillarp) techniques. Neonatal treatment with 6-OH-DA (100 mg/kg s.c.) or 5,7-HT (50 mg/kg s.c.) causes a marked and permanent degeneration of distal NA or 5-HT nerve terminal projections in the brain (e.g. in the cerebral cortex and spinal cord) as well as a NA or 5-HT hyperinnervation in regions close to the NA or 5-HT perikarya in the brainstem (a pruning effect). The 6-OH-DA treatment produces in addition a permanent sympathectomy. Administration of GM_1 alone (4x30 mg/kg s.c., 24 hr interval) to newborn rats had no significant effect on the postnatal development of the NA and 5-HT parameters analyzed. Neither were any effects on the central dopamine neurons observed. It was also found that GM_1 treatment did not appear to have any detectable effects on the 6-OH-DA and 5,7-HT induced alteration of the central NA and 5-HT neurons when analyzing the animals at the age of one week. These findings suggest that GM_1 treatment did not interfere with the primary neurodegenerative actions of 6-OH-DA and 5,7-HT. At the age of one month there were signs of a counteracting effect of GM_1 on the 6-OH-DA and 5,7-HT induced changes of NA and 5-HT neurons respectively. This effect of GM_1 was most clear-cut with respect to the 5,7-HT induced 5-HT nerve terminal degeneration in the cerebral cortex where it was found that the endogenous 5-HT levels were 72% (frontal cortex) and 41% (occipital cortex) of control after 5,7-HT + GM_1 treatment as compared to 26% and 20% respectively after 5,7-HT treatment alone. It was furthermore observed that GM_1 treatment had a moderate counteracting effect of the 6-OH-DA induced sympathectomy, noted both in the iris and the superior cervical ganglion. Taken together, the present results suggest that GM_1 ganglioside treatment may have a degeneration preventing and/or a regrowth stimulatory action on NA and 5-HT neurons damaged by a selective neurotoxin in the neonatal stage. (Supported by MRC (04X-2295), Expressen Prenatal Res. Foundation, Bergvalls and Jeanssons Foundations.)

226.7 FETAL CORTEX GROWTH AND FUNCTION IN DEGENERATING ADULT PERIPHERAL NERVE. Yipeng Tang* and Jerald J. Bernstein. Lab. of CNS Injury and Regen. Res., VA Med. Ctr. and George Washington Univ. Sch. of Med., Dept. Physiology and Neurosurgery, Washington, D.C.

Fetal cortex (E11, 12, 15) implanted into crushed, perineurial minced sciatic nerve grows, differentiates, and matures for up to 120 days. Host peripheral nerves grow through the implant and reinnervate the foot. The following experiments in the rat (Wistar, 300 gms) determine if E11 fetal cortex implants can survive in degenerating peripheral nerve and perhaps innervate denervated muscle. Twenty (5 per group, 7, 14, 21, 30 DPI) rats had sciatic nerve transection at midthigh, the proximal stump tied, reflected and sutured into muscle. The distal stump was tied. The second nerve to the biceps femoris muscle was crushed, a slit made in the epineurium and the perineuria minced with scissors. E11 gestation females had the fetuses removed and placed in complete Tyrode-Ringer solution. A 1.0 mm cube of fetal cortex was placed into the adult host epineurium with a jewlers forceps. Prior to intracardic perfusion, all proximal and distal sciatic stumps and implanted biceps femoris nerves were stimulated electrically (5V., 0.2 msec. 1.0 Hz). Cortical neurons were observed ultrastructurally for the duration of the experiment. Implant growth was poor. The neurons observed had mature nuclei, immature cytoplasmic organelles and were predominantly unipolar. A neuropile was present which contained axodendritic synapses. Macroglia, Schwann, fiboblast and connective tissue sheath cells were observed. In two cases (30 DPI) stimulation of the implanted biceps femoris nerve resulted in weak biceps femoris muscle contraction, whereas stimulation of both sciatic stumps did not. Ultrastructurally, these implants had neurons, a neuropile, the distal biceps femoris nerve contained nerve fibers and the muscle had reinnervated and degenerated end plates. These data show that E11 cortex implants can survive 30 DPI in degenerating peripheral nerve and innervate muscle. (Sponsored by the Department of Navy, ONR, CB-030-82050 and the Veterans Administration).

226.8 BIORESORBABLE NERVE GUIDES BRIDGE TRANSECTED OPTIC NERVE. R. Madison[1] & R.L. Sidman[1], T.H. Chiu*[2], and E. Nyilas*[2], Departments of Neuroscience and Neuropathology, Childrens Hospital and Harvard Medical School[1], and Instrumentation Laboratories, Inc.[2], Boston, MA. 02115.

Nontoxic bioresorbable "nerve guides" (synthetic L-polylactates) were implanted to bridge the intracranally transected optic nerve in adult rats. A 1.5 mm length of the guide (0.75mm I.D., 1.10mm O.D.) was filled with a collagen matrix (bovine; 95% Type I, 5% Type III; Vitrogen, Flow Laboratories) containing 0.5 mg/ml fibrinogen (bovine, Cal Biochem) and 0.4 mg/ml fibronectin (bovine, Kor Biochemicals), and was then implanted, with both the distal and proximal nerve stumps inserted into the guide. Animals were killed 4 (N=1), 6(N=2), and 12 (N=2) weeks following surgery and processed for transmission electron microscopy (TEM).

Four weeks after implantation the "nerve guide" lumen contains a cable of tissue throughout its length. This tissue is well vascularized (see Greatorex, et. al. this volume). TEM shows fibroblasts, macrophages,astrocytes, oligodendrocytes, Schwann cells, numerous unmyelinated axons and a few isolated myelinated axons to be present in the guide. Cable diameter is approximately 0.05 mm.

Six weeks after implantation cable diameter has increased to 0.18 mm. The previous loose stromal network has changed to a more densely packed fibrous bundle containing numerous unmyelinated and isolated small myelinated fibers. Since peripheral sympathetic fibers will invade the central nervous system (CNS) following both nonspecific and specific CNS lesions (Madison and Davis, Exp. Neurol. 80 (1); 167-177, 1983), the possibility that the unmyelinated axons in the guide originated peripherally was tested by performing bilateral superior cervical ganglionectomies (SCGX) 3 weeks prior to the sacrifice of the 12-week animals.

Cable diameter at 12 weeks had enlarged to 0.24mm. The persistence of unmyelinated axons following SCGX is strong evidence that many of these axons have a central origin. At 12 weeks several small (<1μ) myelinated fibers are dispersed throughout the cable as are several axons that are in the process of becoming myelinated. Studies are in progress to unequivocally identify the source of the axons found in the guide.

The mamalian optic nerve and nontoxic bioresorbable "nerve guides" should prove useful for understanding principles of neuronal plasticity and regeneration in the adult CNS. Supported by NIH grants NS14768, N007017, and HD06276.

226.9 PNS GRAFTS ENHANCE AXONAL ELONGATION FROM NEURONS IN FETAL MESENCEPHALON TRANSPLANTED INTO THE ADULT RAT BRAIN. A. Aguayo, A. Björklund* U. Stenevi*and T. Carlstedt*.Neurosciences Unit, McGill University, Montreal,Canada; Department Histology, University of Lund, Lund, Sweden; Department of Anatomy, Karolinska Institute, Stockholm, Sweden.

Recent transplantation experiments have demonstrated the feasability of a) replacing neuronal populations with exogenous nerve cells (Björklund & Stenevi, Physiol. Rev. 59: 62, 1979) and b) enhancing the growth of axons by grafting nonneuronal components of the PNS into the injured brain (Benfey & Aguayo, Nature 296: 150, 1982). We now report the successful combination of both these techniques in the same animal.

METHODS: Host animals. In female Sprague-Dawley rats weighing approximately 250 g, the right nigrostriatal pathway was damaged by a stereotactic injection of 6-OH-DA (8 μg free base in 4 μl, 0.2 mg/ml ascorbate saline). Fetal neuronal grafts. From 6 to 8 weeks later a portion from the ventral mesencephalon of rat embryos was placed intracranially over the right superior colliculus of the host brain stem. PNS grafts: At the same time, a "bridge" linking the fetal neuronal implant and the host striatum was made by transplanting a segment of heterologous sciatic nerve. This "bridge", 2 to 3 cm in length, was placed extracranially beneath the scalp. Through burr holes in the skull, one end of the "bridge" was placed in contact with the fetal implant while the other end was inserted into the caudate nucleus. Fetal and PNS grafts were from rats of the same inbred strain as the hosts.

RESULTS: Histologic examination for dopaminergic fluorescence three months after grafting revealed that PNS grafts were innervated by monoaminergic neurons from the mesencephalic implant. Axons from grafted nerve cells extended along the entire PNS "bridge" and reached the previously denervated caudate nucleus of the host brain. Fluorescent axons showed minimal branching in their long course through the PNS "bridges" but formed dense, albeit short, terminal arborizations within the CNS regions they reached.

CONCLUSIONS: 1. Combinations of fetal neuronal and PNS grafts have been used in vivo to provide both a source and a substrate for axonal growth. 2. Implanted monoaminergic neurons replacing damaged nerve cells in the host brain were shown to have grown axons the entire length of the 2 to 3 cm PNS conduits to reach the striatum, a normal target of nigral projections. 3. Conditions extrinsic to the growing axons appear to influence both fiber elongation and terminal branching.

226.10 DAMAGED OLFACTORY BULB NEURONS REGENERATE AXONS INTO PNS GRAFTS. Beth Friedman and A.J. Aguayo, Neurosciences Unit, The Montreal General Hospital, Montreal, Quebec, Canada.

Peripheral nerve segments grafted into different regions of the rat brain and spinal cord are innervated by CNS neurons but it is not known if such innervation is due to collateral sprouting from nerve cells that retain their normal projections or if it arises by the regrowth of interrupted fibers. Because mitral cells, which normally project to the olfactory cortex via the lateral olfactory tract (LOT) have been shown to grow axons along PNS grafts (Friedman and Aguayo, '82) we have now attempted to determine whether such neurons still innervate the olfactory cortex by using the retrogradely transported fluorescent dyes True Blue (TB), a cytoplasmic label, and Nuclear Yellow (NY), a nuclear tag, to label these projections.

In 11 adult Sprague Dawley rats, an approximately 1 cm long autologous segment from the sciatic nerve was inserted into the olfactory bulb. From 8-20 weeks after grafting, a 3% solution of either TB or NY was placed in gelfoam onto the cut end of the extracranial portion of the graft for one hour; 48 hours later, gelfoam soaked in the second (different) dye was inserted and left in a transection made in the LOT. The rats were perfused transcardially with chilled saline followed by fixative. The brain was removed and tissue sections cut at 25 μm were examined by epi-illumination fluorescence.

In 5 of the grafted olfactory bulbs application of dye (TB or NY) to the graft produced labeled neurons in the olfactory bulb. These could be identified as mitral cells on the basis of cell shape (with TB) or position of the labeled profile in the mitral cell layer (with NY). The most striking finding is that 99% of these neurons which innervate the graft were not labeled by the dye placed in the LOT (202/204 neurons). On the other hand, many of these neurons were adjacent to mitral cells that had failed to innervate the graft but were singly labeled by the dye placed in the LOT, an indication that these cells still projected to the olfactory cortex.

Thus almost the entire population of mitral cells that innervated PNS grafts lacked projections to the olfactory cortex. This suggests that the innervation of peripheral nerves grafted to rat olfactory bulb arises from the growth of projection axons that were interrupted at the time of grafting and not from the extension of axon collaterals of cells that maintained their normal connections.

226.11 GAD IMMUNOREACTIVITY IN TRANSPLANTED MOUSE NEOCORTEX. L.M. Smith, M.F. Bear, D.E. Schmechel* & F.F. Ebner. Div. of Biology and Medicine, Brown Univ., Providence, RI 02912 & Div. of Neurology, Duke Univ., Durham, NC 27706.

Embryonic neocortex transplanted into that of an adult host will survive and develop many features of normal cortex. Our ultrastructural observations have revealed numerous flat-symmetrical type synapses in the transplants. Because synapses with this morphology have been shown to contain GAD in normal rodent cortex, these data suggested that the transplants were richly innervated by GABAergic neurons. We have investigated this possibility further with immunocytochemistry using the GAD antiserum developed by Oertel et al. (1981).

Transplants were made by dissecting rectangular solids (.5x.5x4 mm) of neocortex from embryonic day 14 (E-14), E-17, and PND-0 donors and inserting them with a square capillary tube into the left parietal cortex of adult BALB/cj mice. The host animals were allowed to survive from 14 to 340 days before they were perfused with 10% formalin in saline containing 0.5% zinc salicylate. 30 um vibratome sections were reacted for immunocytochemistry according to the PAP method of Sternberger.

Immunoreactive cells, processes, and puncta were seen in the host cortex and in the transplants of all donor ages. The GAD-positive cells in the host cortex were multipolar or fusiform in shape and appeared evenly distributed in all cortical layers including some cells in layer I. Numerous puncta were also seen in all layers, with the highest density in layer IV. Abnormally high densities of GAD-positive puncta were frequently found in the middle layers of the host cortex near the transplant. Interestingly, the density of puncta was also markedly increased at all survival times in the region of the contralateral hemisphere homotopic to the transplant. The E-14 transplants grew to a relatively large size and contained a high density of Nissl-stained cells. However, GAD-positive neurons were virtually absent from E-14 cases and the puncta were of smaller caliber and fewer in number than at any other donor age. E-17 transplants grew to a moderate size and contained Nissl-stained cells, GAD+ neurons and GAD+ puncta in densities almost equivalent to the surrounding host cortex. PND-0 transplants were always small in volume, but developed immunoreactive neurons and puncta equivalent to the E-17 donors.

These results demonstrate that (1) GABAergic neurons can survive and differentiate in neocortical transplants, (2) the development of GAD+ cells and puncta varies as a function of donor age, and (3) Some GABAergic cells in the host brain respond to the transplantation procedure by local sprouting.

(Supported by NIH grant NS-13031)

226.12 THE DEVELOPMENT OF PEPTIDERGIC NEURONS IN CORTICAL AND HYPOTHALAMIC TRANSPLANTS INTO ADULT MOUSE CORTEX. F. F. Ebner, J. A. Olschowka and D. M. Jacobowitz. Div. of Bio. and Med., Brown Univ. Prov., R.I. 02912 and Lab. of Clin. Sci., NIH-NIMH, Beth.,Md.20205

Neurons from embryonic neocortex and hypothalamus will survive transplantation into the neocortex of adult mice. It is unknown whether the transplants develop the types of neurons found in their source areas. We were also interested in whether peptide-containing processes would grow across the interface into and out of the transplants. We have studied the development and distribution of peptide-containing processes and cells using immunocytochemical localization of the four peptides demonstrated to date in neocortex (vasoactive intestinal polypeptide, VIP; cholecystokinin CCK; pancreatic polypeptide, PP; and somatostatin, SST), as well as five peptides demonstrated in the normal hypothalamus, but not in neocortex (alpha-melanocyte stimulating hormone, MSH; arginine-vasopressin, AVP; corticotropin-releasing factor, CRF; beta-endorphin, END; and substance P, SP).

Neocortical and hypothalamic tissue for transplantation was taken from embryos at 13-16 days of gestation and implanted by capillary tube into the neocortex of 22 adult BALB/cj mice. Neocortex was placed in the left hemisphere, and hypothalamus from the same embryo into the right hemisphere. All 22 recipient animals lived for 4-6 weeks. All 44 transplants survived and contained healthy-appearing neurons. For processing, the brains were cut in a cryostat at 16 um, preincubated for 1 hour in PBS with 4% normal goat serum, and for 48 hr at 4°C in one of the 9 different antisera.

In the cortex-to-cortex transplants, the four peptides seen in the host cortex also developed in the transplant; namely, VIP,CCK, PP and SST. No cells or processes were immunoreactive for MSH, AVP, CRF, END or SP. The processes that develop in the transplant appear to be distributed throughout the transplant, but do not course out of (or from the host into) the implant in detectable numbers. In the hypothalamus-to-cortex transplants, immunoreactive processes and/or cells were labelled with all 9 peptides,but the relative densities of label were markedly different. SP processes formed the densest plexus in all hypothalamic transplants.

These results indicate that peptide neurons which normally develop in the donor structure continue to do so when they develop in a host animal's cortex. Peptide-containing processes do not appear to sprout between donor and host tissue.

(Supported by internal and external (#NS-13031) grants from the NIH).

226.13 TRANSPLANTS OF VASOPRESSIN NEURONS INTO NEONATAL BRATTLEBORO RATS: DEFINING THE OPTIMAL DONOR AGE. D.M. Gash, L.B. Dick* and G.J. Boer*. Dept. of Anatomy, University of Rochester, Rochester, N.Y. 14642 and the Netherlands Institute for Brain Research, Amsterdam, the Netherlands.

Over the past five years, our laboratory has investigated the development and function of fetal hypothalamic tissue transplanted into adult Brattleboro rats homozygous for the diabetes insipidus trait. The present study was conducted to determine the survival and viability of fetal and neonatal hypothalamic transplants into five day neonatal Brattleboro rats.

Prenatal transplant material was obtained from timed-pregnant Long-Evans rats at 11-, 13-, 15-, 16-, 17-, 19-, and 20-days post-coitus (dpc) and neonatal tissue from 0- and 5-day old rat pups. Transplants from 11- and 13-dpc embryos were of the ventral diencephalon containing the presumptive Supraoptic Nucleus (SON). Grafts from the older donors contained the SON proper which had been dissected free from a 1 mm thick coronal section through the anterior hypothalamus.

Transplants were introduced into the third ventricle of ether-anesthetized 5-day neonatal Brattleboro pups via a hand-held spinal needle inserted through an incision in the skull at bregma. At about 40 days following surgery, recipients were perfused with Zamboni's fixative and their brains processed for vasopressin and neurophysin thick-section immunocytochemistry.

The results showed a clear demarcation in this model system between the capability for survival of 11-17 dpc grafts (29/30 viable) and grafts of 19 dpc and older tissue (6/24 survived). In addition, all transplants of 11-17 dpc tissue contained neurophysin-positive neurons while these neurons were only seen in two grafts of 19 dpc and older tissue. These data suggest a close correlation between the ability of a neuron to survive transplantation and its stage of development. In the normal rat fetus, the 17th embryonic day is a time of rapid growth and differentiation of the SON (C.D. Sladek, et al., Peptides 1: Suppl. 1, pp. 51-67, 1980). Events occurring at this period include ultrastructural development of the cytoplasmic apparatus for hormone synthesis, detection of vasopressin in the SON by radioimmunoassay, and axonal outgrowth of neurophysin-positive fibers. In addition, noradrenergic innervation of the SON is being established by the 17th fetal day. It appears, from our present results, that isolation of the SON after initiation of axonal outgrowth and/or noradrenergic innervation, which occurs in transplantation of 19 dpc and older SON, may greatly impair survival of magnocellular vasopressin neurons. Neurons not fully differentiated, as in 11-17 dpc tissue, maintain a greater degree of plasticity than older tissue, and are better able to survive the rigors of transplantation under the conditions inherent in this present experiment.

Supported by grant NS 15109.

226.14 REDUCED TRAUMATIC RESPONSE FOLLOWING NERVE TRANSECTION THROUGH THE USE OF POLYVINYL ALCOHOL AND CHLORPROMAZINE. L. de Medinaceli and A. C. Church. Adult Psychiatry Branch, NIMH, Saint Elizabeths Hospital, Wash. D.C. 20032

Two agents, polyvinyl alcohol (PVA) and chlorpromazine (CPZ), were used at the time of transection of the sciatic nerve in the rat. These substances were tested for their efficacy in reducing some of the degenerative processes that occur in the nerve stumps following transection. We felt that by minimizing these post-traumatic events, we could more effectively promote functional recovery (L. de Medinaceli et al., Exp. Neurol., in press).

Given PVA's physico-chemical properties, we hypothesized that it would reduce the swelling that develops at the tips of the nerve stumps after transection. Through the use of photomicrographs and blind raters, it was determined that PVA significantly prevented such swelling. It is felt that this property of PVA leads to an increased precision in the realignment of the nerve tips and thus provides improved results.

The influx of extracellular calcium into an injured axon has been shown to trigger Wallerian degeneration. We hypothesized that calcium's effect was mediated through its activation of calmodulin, and that by antagonizing calmodulin we could interfere with the degenerative processes. Since CPZ has been shown to inhibit calcium-calmodulin binding, we tested the effects of this agent on degeneration. Sciatic nerves were transected and left in situ with the experimental group receiving locally applied CPZ and the controls receiving Ringer's solution for 24 hours. The nerves were then removed, fixed and stained with Bodian's silver protein method. Our preliminary results showed that under the light microscope CPZ retarded Wallerian degeneration during this period. The CPZ-treated nerve had an appearance that was similar to an undamaged nerve. The initial stage of Wallerian degeneration was visible throughout the control nerve. We interpret these results as supporting our hypothesis concerning the role of calmodulin in Wallerian degeneration. It must be emphasized that the use of these two agents is not the only requirement for a good nerve repair since, in our experience, many other conditions must be met in order to obtain consistently good functional recovery.

227.1 CENTRAL DOPAMINE INHIBITION IN THE SINOAORTIC DENERVATED RAT AS MEASURED BY TYROSINE HYDROXYLASE ACTIVITY AND DOPAMINE CONCENTRATION. N. Alexander, Y. Hirata* and T. Nagatsu. U.S.C. Sch. of Med., Dept. of Med. and Tokyo Inst. of Tech., Dept. of Life Chem., Los Angeles, CA 90033; Yokohama 227

Previous studies showed that resection of arterial baroreceptor nerves affects norepinephrine and epinephrine metabolism in discrete brain nuclei; in this study we show that the dopamine (DA) system is inhibited. Male, Wistar rats, 280-330 gms, were used for total sinoaortic denervation (SAD) or for sham operation (SO). Three days after operation rats were decapitated, brains frozen and cut into 300 µm thick sections. Six, 0.5 mm diam. "punch" samples were obtained from both caudate and substantia nigra areas. Tyrosine hydroxylase activity (TH) and DA concentration were measured by high-performance liquid chromatography-electrochemical detection. Results were, SAD vs. SO rats: TH (pmol/min/mg protein) in caudate, 158 ± 26 vs. 327 ± 46 ($p < 0.01$) and in substantia nigra, 386 ± 40 vs. 740 ± 130 ($p < 0.02$); caudate DA (pmol/mg protein), 296 ± 50 vs. 500 ± 70 ($p < 0.05$). In conclusion, this study shows, for the first time, that arterial baroreceptor reflex pathways affect the central DA system. Further studies are needed to determine if impaired arterial baroreflex function contributes to known reductions in DA metabolism of certain disease states.

227.2 QUALITATIVE AND QUANTITATIVE IMMUNOCYTOCHEMICAL STUDIES OF THE DISTRIBUTION OF MONOAMINE NERVE CELL BODIES IN THE FUNCTIONALLY DEFINED SUBNUCLEI OF THE NTS IN THE RAT. K. Fuxe*, M. Kalia, T. Hökfelt*, L. Agnati*, A. Härfstrand* and M. Goldstein. (Spon: C. P. Bianchi). Dept. Histol. Karolinska Inst., Stockholm 104 01 and Depts. Neurol. and Pharm. Thomas Jefferson Univ., Phila., PA 19107.

Previous studies have demonstrated the existence of monoamine containing cell bodies in the dorsal medulla including the nTS. However, there is no information available with regard to the distribution of these cell bodies in relation to the functionally distinct subnuclei of the nTS. In 25 adult, colchicine treated rats (180µgi.c.), the distribution of monoamine containing neuronal perikarya in the nTS was studied with immunoperoxidase and immunofluorescence techniques using antibodies raised against TH, DBH & PNMT. The sections were counterstained with cresyl violet after intial photography to determine the location of the cytoarchitectonically distinct subnuclei of the nTS. The results are summarized in Table 1. The A2 monoamine cell group (Dahlström & Fuxe'64) was localized in the mnTS,ncom and dmnX and contained TH and DBH positive nor-adrenergic neurons. In addition adrenergic neurons (PNMT positive) were identified in the periventricular region (PVR) and dorsal parasolitarius region (dPSR). Thus, there appears to be a differential distribition of monoamine neurons in the nTS. The mnTS which has been implicated in gastrointestinal function (Kalia and Mesulam'80a) contains noradrenergic neurons and the dPSR and PVR which are associated with cardiac and baroreceptor activity (Kalia and Kropilak '81; Katz and Karten '79, Kalia and Welles '80) contain adrenergic neurons. This suggests that monoamine neurons in the nTS have different roles in central autonomic regulation. Supported by HL 30991, Am.Hrt.GRT.81-978, Swedish Med. Res.Grt.14X-04246-10B .

TABLE 1: Distribution of TH, DBH and PNMT positive cell bodies in the nTS and adjacent regions of the dorsal medulla

level		ncom	mnTS	PVR	dPSR	vPSR	ap	dnmX
0.6	TH	-	±	+	±	±	-	±
and	DBH	-	±	1.5+	±	±	-	±
0.4	PNMT	-	±	1.5+	+	±	-	±
	TH	-	+	1.5+	++	±	-	+
0.1	DBH	-	+	+	+	±	-	+
	PNMT	-	±	+	±	±	-	±
	TH	±	++	-	1.5+	±	++	±
-0.2	DBH	±	++	-	+	±	++	±
	PNMT	0	0	-	±	±	0	0
	TH	+	++	-	0	±	++	+
-0.5	DBH	+	++	-	0	±	++	+
	PNMT	±	0	-	0	0	0	+

227.3 DISTRIBUTION OF NEUROPEPTIDE NERVE TERMINALS WITHIN THE SUBNUCLEI OF THE NUCLEUS OF THE TRACTUS SOLITARIUS OF THE RAT. M. Kalia, K. Fuxe* and T. Hökfelt*. Dept. Neurol. Thomas Jefferson Univ. Phila. PA 19107 and Dept. Histol. Karolinska Institutet, Stockholm 104 01 Sweden.

The location of substance P, enkephalin, somatostatin (SRIF) and neurophysin II immunoreactive nerve terminals and preterminal processes in the nucleus of the tractus solitarius (nTS) and the adjacent regions of the dorsal medulla was examined using the indirect immunofluorescence method combined with cytoarchitectural identification of subnuclear groups in the same section. In 22 adult Sprague-Dawley rats 14 µm thick serial cryostat sections were incubated with antisera raised against substance P, enkephalin, SRIF and neurophysin II. All four peptides were examined in adjacent sections at five different rostro-caudal levels in the medulla, from 1mm caudal to the obex to 2 mm rostral to the obex. The sections were photographed and then counterstained with cresyl violet so that the location of the immunoreactive nerve terminals could be related to cytoarchitectonically distinct subnuclei. The vlnTS, vnTS, ni and nI (the subnuclei associated with respiratory function) showed moderate density of SRIF, scattered substance P and enkephalin and no neurophysin II immunoreactive nerve terminals. The dlnTS, dnTS and ncom (the subnuclei associated with cardiac and baroreceptor function) showed moderate density of neurophysin II, substance P and enkephalin immunoreactive nerve terminals. The mnTS (the subnucleus associated with gastrointestinal function) showed moderate amounts of substance P and weak immunoreactivity for the other three peptides. The densest collection of peptide immunoreactivity however, was found outside the nTS in the adjacent periventricular region (PVR), dorsal and ventral parasolitarius regions (dPSR and vPSR). These regions represent the sites where the dendrites in the adjoining nTS subnuclei project. The vPSR contains moderate density of substance P and enkephalin immunoreactivity; the dPSR contains enkephalin immunoreactivity in moderate amounts and the PVR contains substance P and enkephalin immunoreactivity implicating these peptides in respiratory, cardiovascular and gastrointestinal modulatory function respectively. Since diffferent peptides were found in the somatic and dendritic regions of a given cell group it can be concluded that a single neuropeptide might be serving different functions on a given class of nTS neurons. These different effects of neuropeptides might be responsible for the integrative functions involved in central autonomic regulation.

Supported by: USPHS Grant HL-30991 and American Heart Association Grant 81-978 to MK, Swedish Medical Research Council Grants 14X-04246-10B to KF and 04X-2887 to TH.

227.4 A SLICE PREPARATION OF THE RAT NUCLEUS TRACTUS SOLITARIUS (NTS): NEURONAL ACTIVITY AND RESPONSES TO NEUROPEPTIDES. G.B. Siggins[1], J. Champagnat*[2], L.Y. Koda[1], and M. Denavit-Saubie[2]. [1]A.V. Davis Ctr., The Salk Inst., P.O. Box 85800, San Diego, CA 92138 and [2]Lab. Physiologie Nerveuse, Dépt. Neurophysiologie appliquée, C.N.R.S., Gif-sur-Yvette, France 91190.

The NTS plays a key role in cardiovascular and respiratory function. Vagal and glossopharyngeal afferents in the tractus solitarius (TS) terminate in the NTS, where they evoke excitatory effects. The NTS contains many putative transmitters, including several neuropeptides. We have developed an in vitro slice preparation of the NTS to help clarify the synaptic interactions in the NTS and the role these peptides may play. Albino rats (100-170g) were decapitated at the upper cervical spinal cord. The brainstem was removed and placed in cold (4°C) physiological salt solution (PSS) gassed with 95% O_2, 5% CO_2. Coronal slices (350-500 um thick) were cut on a tissue chopper, placed in PSS in the recording chamber and then completely immersed and continuously superfused with PSS (31-35°C) at 1-2 ml/m. We recorded single units and stimulated the TS with a concentric electrode. Most extracellularly recorded neurons (70%; n = 110) discharged spontaneous spikes at 0.5-10/s. Sixteen neurons were recorded intracellularly; most of these were silent. Membrane potentials of -50 to -70 mV and evoked action potentials of 50 to 100 mV were recorded; input resistances of 20 MΩ were estimated. Forty of 70 neurons tested were activated by TS stimulation with 3-10 ms latencies to a single spike. This driven activity is probably orthodromic because: 1) spike latencies varied by several ms with low frequency stimulation; 2) collision tests were negative; 3) intracellularly, evoked spikes always arose from an excitatory postsynaptic potential. Inhibitory phenomena also appear in the slice. Spikes evoked by TS stimulation were followed by a 30-100 ms silent period, during which a second stimulus failed to evoke a spike. In some intracellular recordings, flurries of small, spontaneous depolarizations indicative of inverted inhibitory postsynaptic potentials appeared after injection of chloride. Superfusion of Substance P (0.01-1 nM) increased the firing rate of 8 of 10 neurons tested. Met-enkephalin (1 uM) decreased the firing rate in 3 neurons, and had no effect in another 4 cells. Somatostatin 28 (0.1-1 µM) decreased firing in 5 of 5 cells. We believe this preparation will be useful for study of synaptic transmission in the NTS, where many amines and neuropeptides may play critical roles in cardiovascular and respiratory function. Supported by C.N.R.S. (#3586 and 3281), D.G.R.S.T. (#82.72128), Fondation pour la Recherche Medicale, and USPHS (HL 25457, DA 01785, AM 26741).

227.5 MICRO-INJECTION OF SOMATOSTATIN RELATED PEPTIDES INTO THE RAT NUCLEUS TRACTUS SOLITARIUS: BLOOD PRESSURE. L. Y. Koda, N. Ling*, C. Bakhit, S. G. Madamba* and F. E. Bloom. The A. V. Davis Center and Neuroendocrinology Laboracory, The Salk Institute, La Jolla, CA 92037.

The nucleus tractus solitarius (NTS) provides a model to examine the functional role of central peptidergic and monoaminergic neuron systems in the regulation of blood pressure. To examine the possible role of somatostatin in cardiovascular regulation, we have micro-injected various peptide fragments of somatostatin 28 (SS28) into the area of the NTS. Male Sprague Dawley rats (150-250g) were anesthetized (pentobarbitol), and placed in a sterotaxic apparatus. Blood pressure was measured from the abdominal aorta. Various concentrations of SS28, peptide fragments of SS28 or yohimbine were injected into the caudal NTS. Injection of vehicle (500nl/10s; 50-80um diameter pipette) alone was followed by a small (2mmHg) decrease in blood pressure. SS28, SS28(1-12) and SS28(15-28) (somatostatin 14) all caused an immediate and statistically significant decrease in blood pressure as compared to vehicle. SS28(1-10) had no significant effect. SS28 was tested at three doses (2.5, 25 and 250pmols); the other peptides were tested only at the 250pmol dose. The low dose of SS28 caused an 8mmHg drop in blood pressure, the medium dose, a 10mmHg drop and the high dose a 16mmHg drop. SS28(1-12) caused a significantly ($p<.01$) less drop in blood pressure (6mmHg) when compared to either SS28 or SS28(15-28) (23mmHg). Since the injection of catecholamine analogs into the NTS or locus coeruleus has been reported to decrease blood pressure, we examined the effect of SS28 on the in vitro release of tritiated norepinephrine (*H-NE). Preliminary results indicate that SS28 (500 nM) enhances the potassium (30-50 mM) induced release of *H-NE from both NTS and locus coeruleus slices. Based on these in vitro release results we attempted to block the hypotensive effect of SS28 with a unilateral NTS injection of yohimbine (alpha adrenergic blocking agent) 10 min prior to an ipsilateral NTS injection of 250pmols SS28. Three doses of yohimbine were tested (6.25, 62.5 and 625 pmols). The 6.25 pmol dose caused a brief 20-25mmHg drop in blood pressure but did not block SS28. The 62.5 pmol dose caused a brief 20-40mmHg drop in blood pressure and partially blocked the SS28 hypotensive response. The 625pmol dose of yohimbine had little effect on blood pressure by itself but blocked the subsequent hypotensive dose of SS28. These data indicate that the C-terminal portion of somatostatin is important for the hypotensive potency of intramedullary injected somatostatin related peptides and that catecholamine-peptide interactions may be important to our understanding of CNS cardiovascular control mechanisms. Supported by USPH HL25457 AA07273.

227.6 CARDIOVASCULAR EFFECTS OF A1 LESIONS ARE ATTENUATED BY PRE-TREATMENT WITH INTRACISTERNAL 6-HYDROXYDOPAMINE. M.J. West, J.M. Elliott,* B.H. Stead,* and J.P. Chalmers*. (SPON: C. Straznicky). Centre for Neuroscience, Flinders University, South Australia, 5042.

Electrolytic lesions in the ventrolateral medulla coinciding with the A1 group of norepinephrine cells (A1 lesions) produce acute hypertension and bradycardia (Blessing, W.W., West, M.J., & Chalmers, J.P., Circ. Res., 49: 949, 1981). In the present study we have investigated the role norepinephrine (NE) cells and their projections may play in mediating these cardiovascular effects, by pretreating rabbits with intracisternal (ici.) 6-hydroxydopamine (6OHDA).

In preliminary experiments, 6OHDA (600 µg/kg free base) dissolved in normal saline (NS), depleted spinal cord NE to less than 1% of control levels whereas the same dose of 6OHDA dissolved in 0.2% ascorbate saline (AS) only depleted spinal cord NE to 24% of control levels. There were no significant differences in depletion of NE in all other brain regions.

Animals which were to be lesioned received ici. injections of 6OHDA (400 µg/kg in AS or 600 µg/kg in NS), or of vehicle alone. One or two weeks later, A1 lesions were made under halothane anaesthesia and arterial blood pressure (BP) and heart rate (HR) were monitored for one hour. A segment of lower thoracic spinal cord was then taken for NE and 5-hydroxytryptamine (5HT) assay before the brain was perfused for histological assessment of the lesions.

The cardiovascular effects of A1 lesions in animals pretreated with 6OHDA in AS (n=7) were not significantly different from those in AS vehicle pretreated animals (n=9). BP increased by 37±8, and 35±7 mmHg and HR fell by 73±11 and 61±11 beats/min respectively. In the animals receiving 6OHDA in AS spinal cord NE was depleted to 23% and 5HT concentrations remained unchanged.

On the other hand, in animals given 600 µg 6OHDA in NS (ici.) spinal NE fell to 2% of control and 5HT levels were again not reduced. The increase in arterial pressure was almost halved (23±7 cf 39±6 mmHg) compared to rabbits treated with NS alone. The bradycardia was also reduced by 50% (-46±8 cf -83±10 beats/min).

These results suggest that depletion of spinal cord NE to 2% of control levels significantly attenuates the cardiovascular effects of A1 lesions. It seems likely therefore that the cardiovascular effects of A1 lesions are mediated, at least in part, by NE projections descending from the brainstem.

227.7 VASOACTIVE INTESTINAL POLYPEPTIDE, BUT NOT SUBSTANCE P OR ACETYLCHOLINE IS THE POTENTIAL CEREBRAL VASODILATOR TRANSMITTER. T.J-F. Lee, A. Saito* and I. Berezin*, Dept. of Pharmacol., So. Ill. Univ. Sch. of Med., Springfield, IL 62708 and Dept. of Neurosci., McMaster Univ., Hamilton, Ontario, Canada.

Cerebral blood vessels of several species have been shown to receive vasodilator nerves. The nature of the dilator transmitter however has not been positively identified. Acetylcholine (ACh), substance P (SP) and vasoactive intestinal polypeptide (VIP) have been shown to be present in cerebral blood vessels. When exogenously applied, these substances have been shown to relax cerebral blood vessels under certain conditions both in vivo and in vitro. The possible role of these substances as vasodilator transmitters in cat cerebral arteries is therefore examined by pharmacological and ultrastructural immunocytochemical techniques. The results indicate that ring preparations of the cat cerebral arteries with intact endothelial cells relax upon application of VIP, ACh and SP at low concentrations but constrict with application of higher concentrations of ACh and SP. In contrast, the cerebral arterial rings without endothelial cells (removed by mechanical rubbing) constrict exclusively upon application of ACh and SP at any concentration examined. These arteries without endothelial cells however still relax upon application of VIP and upon transmural nerve stimulation (TNS). These results suggest that the effect of direct action of ACh and SP on smooth muscle cells is constriction. Both ACh- and SP-induced relaxations depend on intact endothelial cells; presumably they release a dilator substance from the endothelial cells which then causes vasodilation. On the other hand, VIP-induced relaxation is independent of endothelial cells. VIP seems to relax smooth muscle directly. Since the nerve terminals of any type are confined to the adventitial layer of the vessel wall, the wide distance between nerve and endothelium makes it very unlikely that the nerve-released ACh and SP can induce vasodilation indirectly through the endothelial cells. Taken together, these results suggest that VIP acts more like a dilator and SP and ACh more like constrictor transmitters. Results from immunocytochemical studies using the protein A-gold technique indicate that VIP-like immunoreactivity is indeed found in the large granular vesicle in the nerve terminals and axons. The immunoreactivity persisted following chronic sympathetic denervation, indicating that VIP-like substance is present in the granular vesicles of the nonadrenergic nerves. It is therefore suggested that a VIP-like substance, but not ACh or SP, is the potential vasodilator-transmitter in the cat cerebral blood vessels. (Supported by NIH HL 27763, AHA/IHA and Southern Illinois University School of Medicine.)

227.8 ROLE OF THE CHOLINERGIC SYSTEM IN THE CEREBROVASCULAR VASODILATION ELICITED BY STIMULATION OF THE DORSAL MEDULLARY RETICULAR FORMATION IN RAT. C. Iadecola, M.D. Underwood*, T. Ishitsuka, D.J. Reis, Lab of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Electrical stimulation of the fastigial nucleus of the cerebellum (FN) or of the dorsal medullary reticular formation (n. parvocellularis) (DMRF) increases regional cerebral blood flow (rCBF) globally throughout brain. However, the vasodilation elicited by stimulation of these two systems differs in that, outside of primary projection fields, FN increases rCBF independently of local metabolic activity (Nakai et al., Brain Res., 260:35, 1983), while DMRF increases both parameters proportionally (Iadecola et al., Brain Res., in press). Furthermore, we have recently found that cholinergic mechanisms play an important role in the cerebrovasodilation elicited by FN stimulation (Iadecola et al., Neurosci. Abst., 8:424, 1982). In the present study we sought to determine whether the metabolically-linked cerebrovascular vasodilation elicited by DMRF stimulation is also cholinergically mediated.

Rats were anesthetized (chloralose), paralyzed (tubocurarine) and artificially ventilated. Arterial pressure (AP), heart rate, and body temperature were continuously monitored and controlled and blood gases were kept in physiological range. DMRF or FN were electrically stimulated (50-100 uA; 50 Hz; 1 sec on/1 sec off) and AP maintained in the rCBF autoregulated range. rCBF was measured in 13 brain areas by using the 14C-iodoantipyrine technique with regional dissection (Nakai et al., Am. J. Physiol., 243:H226, 1982).

In controls (n=5), rCBF ranged from 62 ± 1 (ml/100 g x min) in corpus callosum to 119 ± 8 in inferior colliculus. Administration of atropine sulphate (0.3 mg/kg i.v.) in unstimulated animals did not change rCBF in any of the regions studied ($p>0.05$ vs. untreated controls). As before, electrical stimulation of DMRF (n=5) or FN (n=5) in untreated rats, increased rCBF globally ($p<0.005$) and maximally in cerebral cortex (DMRF: up to 223% of control in parietal cortex; FN: up to 200% in frontal cortex). In contrast, stimulation of FN in rats pretreated with atropine failed to increase rCBF ($p>0.05$; n=5), while the cerebrovascular dilation elicited by DMRF stimulation was not affected by the drug ($p>0.05$ vs. DMRF stim. in untreated animals; n=5). We conclude that: (a) atropine alone does not affect resting rCBF; (b) unlike FN, the vasodilation elicited by DMRF stimulation in not mediated by muscarinic receptors in brain and/or blood vessels. Cholinergic mechanisms do not seem to be involved in the cerebrovascular vasodilation secondary to increased cerebral metabolic activity. (Supported by Grant HL18974).

227.9 RELAXATION OF ISOLATED BOVINE, PORCINE AND HUMAN BRAIN ARTERIES BY VASOACTIVE INTESTINAL PEPTIDE (VIP) AND PARATHYROID HORMONE (PTH). Y. Suzuki*, D. McMaster*, K. Lederis*, F.E. LeBlanc*, M. Huang* and O.P. Rorstad (SPON: J.P.H. Wyse). Depts. of Pharmacology, Clinical Neuroscience and Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Recent observations suggest that vasoactive intestinal peptide (VIP) may participate in the regulation of brain blood flow. We have studied the relaxant effect of VIP on isolated strips of bovine, porcine and human brain arteries including the middle cerebral artery (MCA, branches distal to the bifurcation), anterior cerebral artery (ACA, pericallosal or callosomarginal branches), posterior cerebral artery (PCA) and basilar artery (BA). VIP (0.3 to 300 nM) elicited a dose-dependent relaxation, in all three species studied, of arteries which had been contracted with prostaglandin $F_{2\alpha}$ or KCl. The relative responsiveness of bovine arteries to VIP was MCA ≃ ACA > BA ≃ PCA. The ED_{50} (relative to maximal vasorelaxation by papaverine) of the VIP effect on all bovine brain arteries studied ranged from 3 to 14 nM. The ED_{50} of the VIP effect on porcine BA and human MCA were 4.5 nM and 1.9 nM respectively. PHI (a peptide having considerable structural homology with VIP) relaxed bovine arteries at a slightly lower potency compared to VIP, whereas the other homologous peptides, secretin and glucagon, were inactive. Interestingly, PHI has considerably more homology with the C-terminal half of VIP than do secretin and glucagon. The fragments VIP-(1-12) and VIP-(10-28) had neither agonist nor antagonist activity on brain arteries, but VIP oxidized at methionine to the sulfoxide (by dilute H_2O_2) or to the sulfone (by performic acid) retained the vasorelaxant effect of the intact peptide. Native bovine parathyroid hormone (bPTH)-(1-84) and synthetic bPTH-(1-34) potently relaxed the bovine MCA and BA, porcine BA and human MCA with the following ranges of ED_{50} for all the arteries studied: bPTH-(1-84), 11 to 23 nM; bPTH-(1-34), 9 to 14 nM. Pretreatment of arterial strips with a PTH inhibitor, [Nle^8,Nle^{18},Tyr^{34}] amide, attenuated the vasorelaxant effect of both PTH preparations. In conclusion: (i) VIP and PTH relax a variety of brain arteries from cattle, hogs and humans. The potencies of the vasorelaxant effect of VIP and PTH are consistent with the potencies for stimulation of adenylate cyclase by these two peptides (Huang and Rorstad, unpublished observation); (ii) The structure-activity relationships for VIP relaxation are complex; and (iii) The bovine arteries from the anterior part of the circle of Willis are more responsive to VIP than those from the posterior part.
(Supported by the MRC of Canada and the AHFMR).

227.10 CHARACTERIZATION OF THE PEPTIDE RESPONSIVENESS OF ADENYLATE CYCLASE IN BRAIN ARTERIES AND CEREBRAL CORTICAL MICROVESSELS. Minta Huang* and O.P. Rorstad, Dept. of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

An increasing body of evidence suggests that peptides may participate in the regulation of cerebral blood flow, possibly by vasodilation. On the basis that cAMP appears to be an intracellular messenger contributing to vasodilation by some vasoactive agents, we had previously studied the effect of several peptides, many of which are vasoactive, on vascular adenylate cyclase (AC). We have reported elsewhere that vasoactive intestinal peptide (VIP) and bovine parathyroid hormone (bPTH)-(1-34) potently stimulate AC in rat cerebral cortical microvessels with ED_{50} values of 100 nM and 6.3 nM respectively. We have subsequently extended our study to a broken cell preparation of bovine brain arteries consisting of branches from the circle of Willis and the basilar artery. AC in these arteries was stimulated by VIP, bPTH-(1-34) and PHI (a peptide having considerable structural homology with VIP) with respective ED_{50} values of 10 nM, 30 nM and 100 nM and respective efficacies of VIP ≳ PHI > bPTH-(1-34). These results are consistent with the observed potencies of VIP and bPTH-(1-34) for relaxation of isolated helical strips of bovine brain arteries (Suzuki, Lederis, Huang and Rorstad, unpublished observation). Combination of maximally active concentrations of VIP and PHI did not result in enhancement of the arterial AC response over that observed with either peptide alone. In contrast, combination of a maximally active concentration of bPTH-(1-34) with either VIP or PHI produced a quantitatively additive effect on arterial AC activity. These additivity studies agree well with our previous observations using cerebral cortical microvessels, consisting of capillaries, arterioles and venules. That is, maximally active concentrations of VIP and bPTH-(1-34) produced an additive effect on AC activity and use of a PTH inhibitor antagonized the AC response to bPTH-(1-34) but not to VIP. Interestingly, bovine cerebral cortical microvessels were relatively unresponsive to VIP, in that a 1 μM concentration effected only a 20-30% increase over basal AC activity. In contrast, bovine cerebral cortical microvessel AC actively responded to bPTH-(1-34) with a four-fold stimulation and an ED_{50} of 10 nM. In conclusion, our results suggest that: 1. Brain arterial, in addition to microvascular, AC is activated by VIP and PTH via pharmacologically distinct receptors for these two peptides, and 2. A very considerable difference exists between bovine brain arteries and cerebral cortical microvessels with respect to the responsiveness of their AC to activation by VIP but not to activation by PTH. (Supported by the MRC of Canada and the AHFMR).

227.11 SEROTONERGIC EXCITATION OF SYMPATHETIC PREGANGLIONIC NEURONS. Robert B. McCall. Cardiovascular Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

The intermediolateral cell column is the main site of origin of sympathetic preganglionic neurons (SPNs). This area of the spinal cord receives a dense input from serotonergic-containing neurons located in raphe pallidus, raphe obscurus and ventral areas of the medulla. However, the nature of the interaction between serotonergic neurons and SPNs remains controversial. In the present investigation, the effects of microiontophoretically applied serotonin (5-HT) on the extracellularly recorded discharges of SPNs were studied in anesthetized cats (n=119). Thoracic neurons were identified as SPNs if: 1) the onset latency of an antidromically evoked discharge remained constant over a range of stimulating intensities, 2) the response to stimulation was all or none and exhibited a sharp threshold, 3) the spike followed high frequencies of stimulation and 4) collisions between orthodromically conducted action potentials and antidromically elicited action potentials could be observed. Onset latencies of antidromically elicited action potentials ranged from 14 to 47 ms. Thirty percent of the SPNs were spontaneously active. These cells fired irregularly and had a mean discharge rate of 1.1 spikes/sec. Low ejecting currents of 5-HT (5-30 nA) invariably excited spontaneously active SPNs (n=22). 5-HT also excited the vast majority of quiescent SPNs (n=34), as well as neurons brought to discharge threshold by iontophoresis of the excitatory amino acid L-glutamate (n=7). A population of SPNs was identified which was insensitive to the excitatory effects of both 5-HT and L-glutamate (n=17). In contrast, current controls had no effect on the discharges of SPNs. Iontophoretic (5-10 nA, n=22) or intravenous (0.05-0.2 mg/kg, n=12) administration of the putative 5-HT antagonists methysergide and metergoline blocked the excitatory effects of 5-HT, but not L-glutamate, on SPNs. The blockade of the 5-HT-induced excitation was not associated with a local anesthetic action of methysergide or metergoline. Methysergide and metergoline reduced the firing rate of spontaneously active SPNs in intact animals. Iontophoretic 5-HT also excited SPNs in spinal animals (n=46) and this effect could be blocked by methysergide and metergoline. However the 5-HT antagonists failed to reduce the discharge rate of spontaneously active SPNs in spinal animals (n=8). These data provide strong evidence to support the contention that serotonergic neurons provide a tonic excitatory input to SPNs or to closely adjacent antecedent sympathoexcitatory interneurons.

227.12 EFFECTS OF SPINAL LESIONS ON SUBSTANCE P LEVELS IN RAT INTERMEDIOLATERAL CELL COLUMN: EVIDENCE FOR LOCAL SPINAL REGULATION. B.M. Davis, J.E. Krause, J.F. McKelvy and J.B. Cabot. Dept. of Neurobiology, SUNY at Stony Brook, Stony Brook, NY 11794.

Substance P (SP) has been localized to the neuropil of sympathetic preganglionic neurons (SPN's) in light and electron microscopic studies. Two recent reports have suggested that the majority of SP in the rat intermediolateral cell column (IML, site of SPN's) was contained in synaptic terminals of bulbospinal axons (Gilbert et al., Neurosci., 7:69, 1982; Helke et al., Fed. Proc., 41:1518, 1982). However, previous investigations in our laboratory indicated the presence of major SP spinal-SPN circuitry in pigeon. The present study examined SP levels in rat IML following various combinations of spinal lesions to discern the relative contributions of bulbospinal and intraspinal SP neurons to SP content in the IML.

70 male Sprague-Dawley rats, 60-90 days old, were used. Rat spinal cords were hemisected at spinal level C2 (n=12) or C7 (n=18) or hemisected at C7 and T7 ipsilaterally (n=12). All rats were killed 7 days after surgery except for 6 rats hemisected at C2, which were killed after 14 days. Right and left IML samples from T2-T3 were micropunched (500 um) and collected from lesioned and matched unoperated control (n=24) rats; SP content was measured by radioimmunoassay (RIA). Complete midthoracic (T5) transections were made in an additional 4 rats and the spinal cords processed for PAP immunohistochemistry following a 7 day survival time.

Following transection of thoracic spinal cord, SP-like immunoreactive staining was clearly evident in the IML caudal to the lesion. These SP positive fibers were studded with boutonal-like swellings and appeared normal. Following high cervical hemisection, depletion of SP (RIA measurements) was bilateral and equal in the IML: 25% depletion was observed after 7 days and 35% depletion after 14 days. However, rats which were hemisected at low cervical levels contained normal or elevated amounts of SP in the IML. The IML in double hemisected rats also contained normal amounts of SP.

Since SP remains in the IML following total transection, SP spinal-SPN circuitry must exist. However, depletion of SP following high cervical hemisection suggests the existence of a SP containing bulbospinal pathway to the IML. The observation that SP levels were normal or elevated following low cervical lesions is consistent with previous data which have shown that intraspinal SP neurons compensate for loss of SP in spinal cord (Tessler et al., Brain Res., 191:459, 1981). Sprouting or increased synthesis by intraspinal SP neurons could be responsible, suggesting an important homeostatic mechanism for maintaining SP content within the IML. (Supported by HL 24103 to JBC.)

228.1 DIGITIZED VIDEO ANALYSIS OF GLABROUS SKIN MOVEMENTS IN CROSS SECTION. R. H. Cohen* and C. J. Vierck, Jr. Dept. of Neuroscience and Center for Neurobiological Sciences, Univ. of Florida, Coll. of Med., Gainesville, FL 32610.

Glabrous skin of the fingertips of anesthetized macaques was cut and examined in cross section in order to determine the relationships between surface movements and underlying structures. A variety of simple tactile stimuli were applied to the surface as mechanical deformation was videotaped from a camera mounted on a dissecting microscope. Utilizing a video-to-computer analysis system, precise measurements of the skin surface profile and epidermal-dermal border from individual labelled frames were digitized for computer analysis by tracing the video image with a superimposed video-cursor. Details of underlying structures were enhanced with intra-vital dyes, yielding textbook-like images of glabrous skin. Distances between intermediate ridges, dermal papillae and limiting ridges, accurate to within 10 um, were measured during precisely controlled force and displacement conditions. A 1mm vertical indentation produced by a .5mm bar yielded a 65% decrease in the distance from the skin surface to the trough of a directly underlying intermediate ridge. Structures located more than 1.5mm from the original skin surface remained virtually motionless in the vertical dimension. This is an ideal condition for transmitting strain to superficial cutaneous mechanoreceptors. Preliminary comparision of the mechanical deformation of the superficial layers of the skin with that of the base of the intermediate ridge indicates that the skin is essentially homogeneous. This means that theoretical predictions of receptor activity can be modelled on basic principles of continuum mechanics. The homogeneous nature of the underlying structures of the skin does not support the notion proposed by Cauna (Anat. Rec., 119:449, 1954) that the intermediate ridges float freely in the loose connective tissue, acting as magnifying levers for the transmission of touch stimuli to the receptors beneath. On the contrary, the tips of the intermediate and limiting ridges are relatively fixed in position during vertical deformations.

Supported by grants NS 07261 and MH 15737.

228.2 VIBROTACTILE NEGATIVE MASKING. G. A. Gescheider, Dept. of Psychology, Hamilton College, Clinton, NY 13323, R. T. Verrillo and D. G. Pelli, Institute for Sensory Research, Syracuse University, Syracuse, NY 13210.

Negative masking occurs in audition, vision and vibrotaction when, in the presence of a masker, the test stimulus becomes detectable at intensities below the threshold level of the test stimulus. It is generally accepted that negative masking in auditory psychophysical experiments is an artifact of the definition of the test stimulus. In vibrotaction nonartifactual negative masking has been observed and interpreted to indicate the presence of a true energy threshold at the periphery. This hypothesis was tested psychophysically by requiring the subject to detect a sinusoidal test stimulus centered in a masker having the same frequency. The masked thresholds were measured against a background of continuous, narrow-band noise. The results confirm the finding of true negative masking in vibrotaction and support the hypothesis of a peripheral energy threshold in cutaneous mechanoreceptor systems.

228.3 CUTANEOUS SENSORY MEASURES FOR PATIENTS WITH HAND INJURIES: HAND REIMPLANTATION AND MEDIAN NERVE REPAIR. Sherry L. Berg* (SPON: Marcelle R. Morrison). DeVry Institute of Technology, Irving, Texas 75062.

Two young adult male patients, one with a hand reimplantation of 1 year and 5 months and another with a median nerve injury one month post-repair were examined for two-point threshold, pinprick sensitivity, evidence of sweating and report to hand emersion in beakers containing water at 40°, 35°, 25° and 20°C. Additionally, the Moberg measures of dexterity and tests for tactile identification were recorded as well as hand dynamometer readings to assess return of function. The uninvolved hand was used as an appropriate control.

The smallest two-point threshold for the reimplanted hand was 10mm, while the uninvolved hand showed differential sensitivities ranging from 3 to 10mm with the greatest sensitivity obtained from the tip of the index finger. For the median nerve injury, sensitivity was reduced by at least 2mm overall for the index finger. Pinprick discrimination for the median nerve injury was reduced and "blunt" was more frequently elicited when "prick" would have been the appropriate response. Both subjects demonstrated sweating in the involved extremity. Latency to establish thermoneutrality in the injured hand was increased and both individuals reported enhancement of intensity of thermal sensation for warm and cool emersions.

228.4 60-HZ ELECTRIC FIELDS IN AIR CAN STIMULATE CUTANEOUS MECHANORECEPTORS IN THE CAT. R. A. Jaffe, R. J. Weigel* and D. L. Lundstrom*, Neuroscience Group, Biology and Chemistry Dept., Battelle Memorial Institute, Pacific Northwest Laboratory, Richland, WA 99352.

Chronic exposure to 60-Hz electromagnetic radiation is known to affect the mammalian nervous system in a variety of subtle ways (Adey and Bawin, Neurosci Res Prog Bull 15:1, 1977; Jaffe et al., Bioelectromagnetics 1:131, 1980; Wilson et al., Bioelectromagnetics 2: 371, 1981). The mechanism whereby these effects are produced remains unknown. This mechanism may be of importance not only from an environmental health perspective but also in understanding any possible role for ephaptic transmission in the central nervous system. It is possible to hypothesize that the effects of electric fields are a result of direct interaction between neuronal membranes and either the surface or induced voltage and current. Alternatively, the biological effects could be produced indirectly, as a result of sensory stimulation and the resulting low-level stress. In order to test this alternative hypothesis, the existence of receptors, sensitive to electric fields, must first be demonstrated.

Using single fiber recording techniques in Ketamine anesthetized cats, we examined the responses of 233 receptor units comprising 10 cutaneous receptor categories. The hind foot and lower leg were exposed to a high-strength 60-Hz electric field while recording from dorsal root fibers supplying that region. Receptor units were categorized on the basis of optimal stimulus, stimulus-response characteristics and location. The number of units tested in each category was as follows: rapidly adapting high frequency (33); rapid adapting low frequency (6); type D hair follicle (40); type G hair follicle (71); type T hair follicle (22); rapidly adapting field receptor (15); slowly adapting type I (16); slowly adapting type II (13); slowly adapting type C (8); slowly adapting unidentified (7); and warm receptors (2). Receptor units in 4 categories generated action potentials in response to the presence of an electric field. The most sensitive receptors were the so-called rapidly adapting field receptors, with thresholds as low as 160 kV/m. This value is well below the local field strength used to produce many of the previously reported effects in animals.

In summary, we have demonstrated the existence of peripheral cutaneous sensory receptors capable of proportional transduction of 60-Hz electromagnetic radiation. We propose that chronic stimulation of these and perhaps other (e.g., vibrissa) receptors could account for many of the reported electric field effects in animals.

This work was supported by the U.S. Department of Energy under contract number DE-AC06-76RLO-1830.

228.5 EFFECTS OF TEMPERATURE ON THE PSYCHOPHYSICAL CHARACTERISTICS OF NON-PACINIAN CUTANEOUS MECHANORECEPTOR SUBSYSTEMS. S.J. Bolanowski, Jr., Center for Brain Research, University of Rochester School of Medicine, Rochester, N.Y. 14642, and R.T. Verrillo, Institute for Sensory Research, Syracuse University, Syracuse, N.Y. 13210.

Psychophysical detection thresholds were obtained over a range of sinusoidal frequencies from 15 to 700 Hz using a small contactor (.008 cm^2), which preferentially excites the non-Pacinian subsystems. The responses of these subsystems were selectively enhanced or degraded by the use of carefully controlled (± 0.5°C) skin surface temperatures of 15, 20, 30, 40 and 43°C. The skin surface temperature was maintained by circulating water at the appropriate temperature through hollow chambers of the contactor's surround. The surround is approximately 33 cm^2 in area. All measurements were made on the thenar eminence. The results show several distinct frequency characteristics that are not of Pacinian-corpuscle origin. For example, the 43°C curve for low stimulus frequencies closely follows a U-shaped function thought to be mediated by Meissner corpuscles as based on electrophysiological evidence. Other portions of these same curves seem to suggest involvement of both Ruffini capsules (20°C, low stimulus frequencies) and Merkel cell-neurite (all temperatures, high stimulus frequencies) subsystems. Averaging of the threshold values across the controlled skin surface temperatures produces a flat function similar to the one commonly referred to as mediating the non-Pacinian (NP) system and described in the duplex theory of vibrotaction (Verrillo, R.T., The Skin Senses, edited by D.R. Kenshalo, Thomas, Springfield, Ill. 1968). The results suggest that the NP system is probably composed of more than one receptor type. As pointed out previously for the P system (Bolanowski, S.J., Jr. and Verrillo, R.T., J. Neurophysiol. 48:836, 1982) and again here, it appears that well controlled skin surface temperatures are necessary for the precise measurement of the various subsystems that mediate the sense of vibration.

228.6 EFFECT OF MECHANICAL STIMULUS SPREAD ACROSS GLABROUS SKIN OF RACCOON HAND ON TACTILE PRIMARY AFFERENT FIBER DISCHARGE. Benjamin H. Pubols Jr. Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, OR 97209.

Receptive field (RF) areas of cutaneous mechanoreceptors innervating the glabrous skin of the raccoon's hand are extremely small when measured at absolute threshold, ranging between 0.05 and 4.5 mm sq, with a median value < 1.00 mm sq (Pubols, L., and Pubols, B., J. Neurophysiol., 1973, 36, 1023-1037). Nevertheless, sufficiently intense suprathreshold stimuli may excite these receptors at a distance. In the present experiments, the role of spread of mechanical deformation of skin was investigated. One feedback-controlled mechanical stimulus probe (1 mm tip diameter) was used to indent the skin (constant depth = 1000 µm, constant velocity = 10 µm/msec) at varying distances from the center of a cutaneous RF, while a second probe was used to monitor vertical displacement of the skin at the site of the RF. Simultaneously, single unit action potentials were recorded from slowly adapting (SA) fibers of the median nerve.

As the stimulus probe moves away from the center of a palmar RF, vertical displacement of the RF declines approximately linearly over a distance of 4-5 mm. In contrast, spike discharge rate during the first 1/2 sec of static displacement is a negatively accelerated, decreasing function of distance between stimulus and RF center.

As the stimulus probe moves away from the center of a digital RF, effective stimulus spread is much less, and there may be no discharge when the stimulus is as little as 1 mm away from the RF center.

In a second type of test, palmar skin was indented 1000 µm at a distance of 2 mm from the RF center and displacement at the RF center was monitored. The monitored RF displacement depth and velocity were then duplicated with a stimulus probe centered over the RF. In most instances, displacement by the distant stimulus yielded fewer spikes than an equivalent displacement centered over the RF. In one exception, a presumed SA Type II unit yielded the opposite results.

These results indicate that (1) SA mechanoreceptors of the raccoon's glabrous skin can be excited by suprathreshold stimuli applied at some distance from the threshold RF, and (2) responses to these distant stimuli cannot be accounted for solely in terms of vertical displacements of the RF. Forces acting parallel, as well as perpendicular, to the skin surface must be considered in attempts to understand the contributions of viscoelastic properties of skin to transduction mechanisms in slowly adapting mechanoreceptors. (Supported in part by research grant NS-19487, USPHS).

228.7 SKIN WETTING INCREASES THE SENSITIVITY OF SA MECHANORECEPTORS IN THE GLABROUS FOREPAW OF THE RACCOON. B.G. Turnbull* & D.D. Rasmusson. Dept. of Physiology & Biophysics, Dalhousie University, Halifax, N.S. Canada B3H 4H7.

During preliminary experiments on mechanoreceptor afferents in the raccoon forearm, it was noted that response properties of afferents from the glabrous skin varied greatly depending on the dryness of the skin. Consequently, direct comparisons of afferent responsiveness were made before and after wetting the skin. Single unit recordings of fibres in the ulnar nerve were made using the dissection technique: a small number of fibers were placed across a platinum electrode in a bath of warm mineral oil and the action potentials of individual fibers were identified on an oscilloscope. Four types of mechanosensory fibres were routinely recorded (Pacinian, RA, SAI and SAII fibres) but only the SAI fibres were studied in the present experiments. Once classified, the receptive fields (RFs) were mapped using threshold and suprathreshold stimuli (applied via Semmes-Weinsteim aesthesiometers, Stoetling), first with the skin dry and the immediately following the application of 0.9% saline or tap water onto the RF.

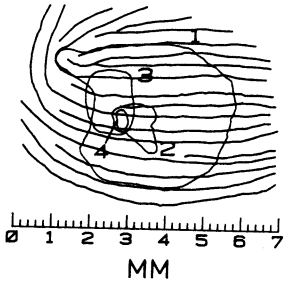
The response threshold was found to be decreased after skin wetting (means: dry, 687 mg; wet, 202 mg). A t-test on the change in thresholds was statistically significant. In no case did skin wetting result in an increase in threshold. In addition to the decrease in threshold, there was an increase in receptive field size determined with any given suprathreshold stimulus.

These findings suggest that skin wetting enhances sensitivity of SAI mechanoreceptors in the raccoon forepaw, although at the possible expense of decreased spatial acuity at the receptor level. However, it appeared that the relative increase in firing was greater at the focus of the receptive field than it was at the RF periphery. More quantitative analysis of RF shape and responsiveness are in progress although the difficulties in quantifying skin wetness are noted. A similar increase in responsiveness was noted in SI cortex of the raccoon following skin wetting (Welker and Seidenstein, 1959). It is possible that this change in receptor sensitivity in the raccoon may be related to the often discussed (and controversial - c.f. Lyall-Watson, Proc. Zool. Soc., 1963, 191, 371) "food-washing" behavior of the raccoon.

Supported by MRC of Canada.

228.8 MAPPING RECEPTIVE FIELDS OF CUTANEOUS MECHANORECEPTIVE AFFERENTS BY MOVING SURFACE STRUCTURES LATERALLY OVER THE GLABROUS SKIN OF THE MONKEY HAND. R.H. LaMotte, L. Becerra-Cabal,* J. Whitehouse,* P. Marks* and J. Ngeow*, Dept. of Anesthesiology, Yale Univ. Sch. of Med., New Haven, CT 06510

By actively touching a smooth surface with the fingertip, humans can detect a single asperity of a very small height - on the order of 1 µm for a raised dot 600 µm wide or a straight edge (step) (R.S. Johansson and R.H. LaMotte, Somatosen. Res., Vol. 1, in press). We recorded evoked responses as these structures were moved across the receptive fields of single quickly-adapting (Meissner Corpuscle) glabrous mechanoreceptive afferents in anesthetized monkeys. The receptive field was mapped with von Frey monofilaments. Then a smooth transparent glass plate on which was etched a 605 µm wide dot or an edge (step) was moved across the receptive field. The area of contact with the skin was viewed through the plate with a microscope and videotaped so that off-line analyses could determine the position of the edge or dot on the skin at the occurrence of each action potential. The minimal asperity height capable of evoking a response was within 1-3 µm of human detection threshold. The receptive field, as shown below for one afferent (located on side of finger tip), differed depending on the type of stimulus used: a von Frey filament delivering 65 mN force (area 1), or 5 mN (area 2) or a glass plate, moving 20 mm/sec across the skin, containing either a single raised dot of 15 µm (area 3) or 3 µm (area 4) height. The greatest discharge was elicited by moving a dot or edge across the ridges, presumably due to greater lateral deformation of the ridges. The response to edges was greater moving against vs. moving off of the edge.



For asperities near threshold, the receptive fields were smaller (e.g. area 4) and obtained only by movement across the ridges. These results indicate the importance of (1) the interaction between geometries of surface microstructures and skin ridge patterns in determining the spatial organization of a mechanoreceptor's receptive field and (2) the role of Meissner afferents in tactile detection of a small asperity on a smooth surface.

Supported by USPHS grant NS15888

228.9 SENSORY RECEPTORS OF THE INCISIVE PAPILLA OF RAT HARD PALATE STUDIED BY PEROXIDASE CYTOCHEMICAL METHODS. Kwan Y. Chan and Margaret R. Byers. Ophthalmology, Anesthesiology and Biological Structure, University of Washington, Seattle, WA 98195.

The incisive papilla (IP) is a pyramid-shaped dome forming the most anterior part of the hard palate in the rat. On each lateral-facing slope of IP is the orifice of the incisive canal, which leads in a dorsal direction to the nasal cavities. Based on studies of serial sections of IP embedded in methacrylate, the medial wall of the canal is heavily populated with chemosensory corpuscles of 40-50 μm diameter, amounting to 30-40 corpuscles within a strip of epithelium 0.3 mm wide, 1 mm long. About a third of these corpuscles are located outside the canal on the lateral slope adjacent to the orifice. The trigeminal origin of the sensory innervation of IP was studied by injecting 2-6 μl of 5% WGA-HRP solution into each side of IP and allowing 24 hr survival for retrograde axonal transport to occur. The rats were perfused with fixative, the trigeminal ganglia (TG) dissected and frozen sections (40 μm) of TG were prepared and processed for peroxidase cytochemistry using the diaminobenzidine or tetramethylbenzidine method. Light microscopy showed that labeled neurons were localized in the lateral half of the maxillary portion of TG, numbering more in the ventral aspect than the dorsal aspect. Based on these data, the TG-derived sensory receptors of IP were further studied by injecting 18-20 μl of 5% WGA-HRP or 30% HRP solution into TG and allowing orthograde axonal transport to occur for 24 hr. The rats were perfused with fixative, the IP dissected, frozen sectioned and processed for peroxidase cytochemistry as above. Light microscopy showed distinct patterns of labeled nerve network at 3 locations: (a) the chemosensory corpuscles, (b) lamina propria of the lateral labium abutting the orifice of incisive canal, and (c) both intra- and subepithelial areas of the dome region of IP. Only the side ipsilateral to the injection site showed labeled nerve elements. Electron microscopy of epon-embedded sections showed that the labeled structures included myelinated and unmyelinated axons, preterminal axons, intracorpuscular (chemosensory) afferent termihals, intraepithelial "free" nerve endings, and various mechanoreceptor-like structures such as lamellated corpuscles in the lamina propria. Within the labeled structures, peroxidase reaction product was localized in vesicular and tubular profiles. Both the orthograde and retrograde labeling techniques were used to analyze and characterize on the ultrastructural level specific differences among the various sensory receptors and in their relationship to the closely associated non-neural elements. These data will be useful in further functional studies of the multiple sensory innervation of hard palate. [Supported by NIH grant # DE05159 and DE00099.]

228.10 LABELING AND NEUROTOXIN DENERVATION STUDIES OF THE MORPHOLOGICAL FEATURES OF THIN-FIBER CUTANEOUS INNERVATION. L. Kruger, B. E. Rodin, Y. Yeh,* S. L. Sampogna* and N. C. Brecha. Depts. of Anatomy, Anesthesiology and Medicine, UCLA Center for Health Sciences, Los Angeles, CA 90024.

The distribution of rat cutaneous nerves was examined in 1) normal animals, 2) following destruction of the thin sensory fiber population by neonatal capsaicin (CAP) treatment, 3) following destruction of the sympathetic postganglionic fibers by surgical extirpation and by neonatal 6-hydroxydopamine treatment, 4) following dorsal root ganglion injection of lectin-conjugated horse radish peroxidase (WGA-HRP) for axonal transport labeling of sensory fibers. Immunohistochemical labeling using antibodies to several peptides suspected of association with sensory nerve was examined with indirect fluorescence (FITC) and peroxidase-antiperoxidase (PAP) methods; some of the latter material was examined electron microscopically (EM).

The most striking finding was the widespread presence of labeled thin sensory fibers penetrating hairy and glabrous skin to below the epidermal stratum lucidum. A substantial proportion of the intraepidermal axons also display selective substance P-like (SP) immunoreactivity, although it is evident from EM observation that most C fibers in any bundle are not SP positive. Elimination of the majority of these intraepidermal axons in CAP-treated animals, but not following sympathectomy, strongly supports the view that these fibers serve a sensory role. Among the more numerous and richer variety of dermal afferent patterns, there is also evidence of thin fibers with SP immunoreactivity arborizing extensively around small blood vessels.

The variety of terminal branching patterns, vesicle and mitochondrial accumulations and peptide distribution remains unclassified, but thin fiber endings are generally distinguishable from most other varieties of cutaneous sense organs. Several morphological markers enable recognition of sensory and sympathetic unmyelinated fibers.

This research was supported by NIH grant NS-5685 and an NRS award to B.E.R.

228.11 CENTRAL PROJECTIONS OF PRIMARY AFFERENT NEURONS THAT INNERVATE THE TEMPOROMANDIBULAR JOINT CAPSULE IN CATS. N.F. Capra and K.V. Anderson. Dept. of Anatomy, Univ. of Miss. Med. Ctr., Jackson, MS 39216.

The receptor population of the temporomandibular joint capsule (TMJ) in cats includes Ruffini endings, paciniform corpuscles, Golgi endings, and free nerve endings. Branches of the auriculotemporal nerve, the deep temporal nerve, and the masseteric nerve contribute to the innervation of these receptors. The perikarya of the primary afferent neurons innervating the TMJ are located in the mandibular portion of the trigeminal ganglion (TG). The present study was performed to identify the distribution and site of termination of the central processes of TG neurons within the trigeminal brain stem nuclei.

The right TMJ was surgically exposed in six pentobarbital anesthetized adult cats. Under an operating microscope, a microliter syringe containing a solution of Horseradish peroxidase conjugated with wheat germ agglutinin (WGA-HRP, Sigma VI, 4% solution) was inserted into the posterior region of the TMJ. A total of 5-10 μl of WGA-HRP were injected into the joint. The animals were allowed to survive from 24-72 hours before euthanization. The animals were perfused with heparinized saline followed by a fixative containing 1.25% glutaraldehyde and 1% formaldehyde in a 0.1 M phosphate buffer (pH 7.40). The fixative was washed out with 0.1 M phosphate buffer solution containing 10% sucrose.

Serial sections of the right trigeminal ganglion and brain stem were sectioned at 50 μm and processed using tetramethylbenzidine (TMB) as a chromagen (Mesulam, 1978). Alternate sections were counterstained with neutral red and studied with bright field and dark field optics.

Labeled perikarya were identified in the mandibular region of TG. Both the peripheral and central processes of many of these cells were clearly labeled. The peripheral processes of the ganglion cells traveled in the mandibular division of the trigeminal nerve. Although most of these fibers were unbranched, on occasion bifurcation of the peripheral processes occurred before they entered the foramen ovale. The central processes of the TMJ afferents entered the brain stem and ended in the chief sensory nucleus or entered the spinal tract of the trigeminal nerve. Labeled fibers in the spinal tract were dorsally located and terminated primarily in the subnucleus oralis and the rostral part of the subnucleus interpolaris of the spinal nucleus of the trigeminal nerve. In the present series of experiments, fibers could not be traced into the subnucleus caudalis. These findings indicate that neural information from the TMJ concerning jaw position is processed in the rostral part of the trigeminal nuclear complex. (Supported by NIDR Research Grant DE06027)

228.12 THE DEVELOPMENT OF MECHANOSENSORY FUNCTION AND SYNAPTIC MORPHOLOGY WHEN REGENERATING AXONS ARRIVE AT NERVE-FREE MERKEL CELLS IN XENOPUS SKIN K. Mearow and J. Diamond. Dept. of Neurosciences, McMaster University, Hamilton, Ontario L8N 325.

Previous work in this laboratory has shown that the Merkel cell-neurite complex in salamander epidermis is a rapidly-adapting touch receptor. We now know (to be published) from TEM examination of serially sectioned salamander skin, using goniometric techniques, that each of the 2-4 boutons of the single functional axon supplying a Merkel cell has a reciprocal synaptic relationship with the cell. These investigations have now been extended to the skin of Xenopus, in which the Merkel cells occur around the visible openings of the cutaneous gland ducts. A voltage-controlled mechanical stimulator of 10 μm diameter was used to compare the mechanosensory thresholds when the stimulus was applied directly over the gland openings ("on" locations) to those when the stimulus was located between the openings, which are on the average about 50-100 μm apart. The most sensitive points were always the "on" ones, and the results indicated that these represented a single population of rapidly-adapting touch receptors; the locations of these coincided with those of the epidermal Merkel cell-neurite complexes, which therefore appear to have a mechanosensory function like the analogous structures in salamander skin. The preliminary EM findings indicate that in Xenopus too the boutons make reciprocal synapses with the Merkel cells. Our eventual objective is to determine whether reciprocal synapses are involved in the mechanosensory transduction process, and/or the trophic interactions that occur between nerves and Merkel cells.

One approach to understanding the possible significance of these reciprocal synapses is to follow the development of mechanosensitivity when sensory nerves grow into skin, and to see whether there is any correlation with any of the characteristic morphological features of the Merkel cell-neurite complex. These studies are being done in Xenopus, during the reinnervation of denervated skin, and the innervation of new skin that has regenerated in place of a portion excised earlier. Merkel cells were shown to be present in both situations; they survive denervation, and they appear in regenerated limb skin even in the absence of nerves. Ingrowing sensory axons eventually contact these Merkel cells, which thus act as targets for these nerves. Preliminary results suggest that the recovery of discrete touch spots requires that such contacts occur. The mechanosensitivity develops gradually however, and this is associated with the gradual maturation of the synapses at the Merkel cells. We hope to determine whether or not complete physiological recovery requires that there is a full restoration of the reciprocal synapse.

(Supported by N.I.H. Grant NS 15592-02).

228.13 FACTORS AFFECTING MECHANOSENSORY FUNCTION IN AN ISOLATED SALAMANDER SKIN-NERVE PREPARATION C.A. Nurse, M. Holmes* and J. Diamond. Dept. of Neurosciences, McMaster University, Hamilton, Ontario L8N 3Z5.

The mechanosensitivity of the salamander skin is based largely on the irregular distribution of rapidly-adapting touch receptors, whose morphological correlates are the epidermal Merkel cell-neurite complexes (Parducz et al., Neuroscience 2: 511-521). Though we now have evidence for the presence of reciprocal synapses between the sensory nerve endings and the Merkel cells, the relationship of this finding to mechanosensory transduction or its modulation remains obscure. In an attempt to investigate this further we have developed an isolated skin-nerve preparation, in which we monitor quantitatively the variation in mechanosensory thresholds in response to controlled manipulations of the fluid environment. The preparations show remarkable stability over a 20 hr period after repeated mappings in a glucose-supplemented, Hepes-buffered amphibian ringer (containing 1.8 mM Ca^{2+}, 1.6 mM Mg^{2+}); in addition they may be kept in enriched medium for at least 2 days at 4°C while retaining normal mechanosensitivity. The Ca^{2+}-antagonist Co^{2+} (2-10 mM) causes a progressive increase in mechanical thresholds within ½ hr of changing solutions; at the higher doses mechanosensory function is reversibly abolished (i.e. threshold stimulus exceeds 30X control). Within a similar time period the threshold is hardly affected by a 6-fold increase in (Mg^{2+}) or Ca^{2+}-removal, though addition of the chelating agent EDTA to Ca^{2+}-free ringer progressively reduces the mechanosensitivity. Of special interest was the effect of 1 mM La^{3+}, another Ca^{2+}-antagonist which blocks transmission at the neuromuscular junction (nmj). In the isolated skin-nerve preparation 1 mM La^{3+} caused a progressive decrease in mechanosensitivity and finally block, over a period of 3/4-1½ hr; however, in contrast to the nmj there was a progressive recovery towards normal sensitivity over the next 4 hr following La^{3+}-removal. Although many of the fine structural changes in the sensory nerve endings (even after recovery of mechanosensory function) resembled that seen at the nmj (e.g. marked reduction in vesicle population, presence of cisternae and coated vesicles), the Merkel cell appeared hardly affected by the La^{3+}-treatment. These results point to a role for Ca^{2+} near the transduction site but do not as yet distinguish whether the nerve ending or the Merkel cell is the transducer element. Other ionic and drug manipulations are presently being examined by correlative electrophysiological and EM techniques.

Supported by N.I.H. Grant NS 15592-02.

228.14 SYMPATHETIC MODULATION OF SLOWLY-ADAPTING TYPE I MECHANORECEPTORS IN THE CAT. W.J. Roberts, S. Elardo* and K. King*. Neurological Sciences Institute of Good Samaritan Hospital and Medical Center, Portland, OR 97210.

Activity was recorded from single, functionally-identified afferent units in the saphenous nerve of anesthetized cats. Repetitive mechanical stimulation of the receptive field with force steps was used to evoke activity in these units. Sympathetic activity was introduced by electrical stimulation of the ipsilateral lumbar sympathetic trunk (cut centrally) at 10 Hz. Touch domes were marked with india ink and the skin patch removed for histochemical analysis. Frozen sections were reacted with glyoxylic acid and examined for catecholamine fluoresence in or near the touch domes.

The mechanical threshold (step force required to produce firing in 50% of presentations) was tracked before and during 3 minutes of sympathetic stimulation (SS) for all units which failed to become "spontaneously" active during SS. The predominant sympathetic effect was a reduction in threshold. Of 18 units tested in 14 cats, 8 showed a marked reduction of >25% (mean change - 48.2% ± 17.7), 8 showed a slight or negligible decrease in threshold (-12.7% ± 7.2), and 2 showed an increased threshold (+68% ± 9.9). These effects persisted with little diminution throughout 3 minutes of SS. A small increase in the force exerted by the tissue against the probe was often observed to parallel the time course of the change in afferent excitability, however piloerection was rarely observed in the receptive field when examined at 30x magnification.

Seventeen other units in 9 cats became "spontaneously" active during SS. This activity began with a latency of 3 to 15 sec and showed an initial high rate which decreased with time but generally persisted throughout 3 minutes of SS. One of these 17 units was recorded from a male cat; the remainder were in females. Only 5 SAI units recorded in female cats failed to develop this "spontaneous" activity.

Intravenous administration of the α-adrenergic blocking agent phentolamine (2 mg/kg) reduced or blocked these sympathetic actions in all 8 units tested.

Histochemical analysis of 16 touch domes in 7 cats showed no catecholamine fluoresence in association with afferent fibers. This contrasts with earlier positive results for hair receptors (Roberts and Levitt, J. Comp. Neurol. 210, 204-209, 1982).

These sympathetic actions on SAI afferents are likely to be mechanically induced (vascular perhaps) because they are reduced by an adrenergic blocker yet no catecholamine efferents are seen in the touch domes.

228.15 EFFERENT FIBERS FROM BRAIN STEM NEURONS IN THE RAT CAROTID SINUS NERVE. A. Hess and I. Cassady*, Dept. of Anatomy, Rutgers Medical School, UMDNJ, Piscataway, NJ 08854 (SPON: S. Rosner).

The carotid body is innervated by the carotid sinus nerve (CSN) a branch of the glossopharyngeal. It is generally agreed that this nerve contains, by far, mostly sensory fibers. More recent studies using HRP retrograde uptake after severance of the CSN, however, have found brain stem motor neurons with HRP accumulation in the nucleus ambiguus (NA) and retrofacial nucleus (Rf); other studies using this same method have found no such neurons. Previous studies have used cats and report very variable numbers of neurons from cat to cat, even in the same study, or disagree on the distribution and organization of the HRP accumulating neurons. The rat is currently being used frequently for studies of the carotid body, and the contribution, if any, of brain stem efferent neurons to the CSN of the rat is unknown. The CSN of the rat was severed and HRP crystals applied to the proximal stump. In addition, various control studies were performed. The hypoglossal nerve was purposely cut in most of the animals at the same time as the CSN in order to ascertain that uptake of HRP had occurred. A small branch of the vagus nerve runs adjacent to the CSN. This nerve was purposely cut in several animals; the number of HRP cells in rats with tenth branch and CSN cut was compared to the number found after CSN cut only.

Nerve cut	Neuron location & no.	Distribution
CSN, 12	NA, 14; Rf, 4	scattered
CSN, 12	NA, 0; Rf, 10	grouped
CSN, 12	NA, 6	scattered
	Rf, 27	grouped
CSN, 12	NA, 9; Rf, 0	scattered
CSN, 10, 12	NA, 85, Rf, 2	grouped
CSN, 10, 12	NA, 133; Rf, 1	grouped

Accidental or intentional interference with the branch of the tenth nerve results in an increased number of HRP-positive neurons.

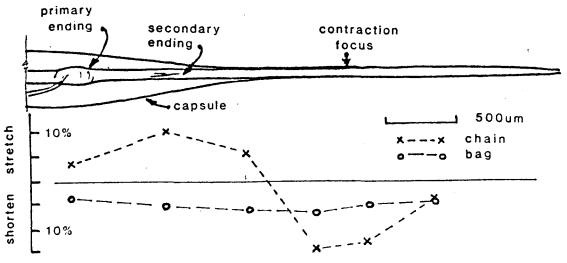
CSN cells tend to occur in rostral portions of the NA and its rostral continuation as the Rf. The distribution of cells is variable from rat to rat: grouped with 4-5 cells at one level in the same section or scattered with only one or, at the most, two cells in each section. In some rats, cells can be restricted to either NA or Rf. Neurons contributing axons to the CSN have not been found in any location other than NA or Rf. No contralateral neurons accumulating HRP have been found.

Because of what appears to be an inherent variability in location and number of brain stem neurons contributing axons to the CSN nerve, more rats are being studied.

228.16 CONTRACTIONS OF CHAIN FIBERS IN ISOLATED CAT MUSCLE SPINDLES. R.E. Poppele, P. Iaizzo* and D.C. Quick*. Lab. of Neurophysiology, Univ. of Minnesota, Minneapolis, MN 55455.

Contraction of intrafusal muscle in spindles modifies the behavior of spindle sensory endings. Activation of fusistatic fibers may cause contractions in chain and bag2 fibers, whereas fusidynamic activation effects only bag1 fibers. We have examined the local changes of length in intrafusal muscle that result from fusimotor stimulation in isolated spindles. Measurements of strain (change in length per unit length) were made from motion picture records of different regions of a spindle during nerve stimulation. In the example illustrated below, stimulation at 80/sec evoked a contraction (local shortening) of chain fibers in one pole of an isometric spindle. The contraction pulled the sensory endings toward that pole and shortened that half of the spindle. The focus of contraction was about 2 mm from the primary ending where chain fibers shortened by as much as 15%. Closer to the sensory region, the same fibers were stretched by as much as 11% with the maximum stretching occuring in the region of the secondary ending. A bag fiber, which we classified as a bag1, appeared to be unloaded by the chain contraction, showing a 3-5% shortening along its entire length in that pole. The findings indicate a loose lateral coupling between bag and chain fibers and suggest that the reduction in dynamic index of the primary sensory response often seen with fusistatic stimulation may be due to a reduced stretch on bag1 fibers.

Supported by Grant BNS 81-19871 from the NSF.



228.17 LACK OF COMPARTMENTALIZATION OF THE HUMAN JAW STRETCH REFLEX. Anne Smith*, Carol A. Pratt, and Christopher A. Moore*. Department of Audiology and Speech Science and Physical Education; Purdue University, West Lafayette, Indiana 47907.

Downward "step" displacements of the human mandible produce large, transient, short latency (8 ms) EMG responses in jaw-closing muscles. These transient EMG responses, in turn, produce large increases in jaw-closing force. Based upon estimates of the reflex stiffness of the human jaw-closing system, Cooker et al. (J. Physiol., 308: 61-78, 1980) suggested that the monosynaptic jaw stretch reflex is a potent or "high gain" pathway that can make a significant contribution to the stability of the mandible.

In contrast, Appenteng et al. (J. Physiol., 179: 409-423, 1978) suggested the monosynaptic jaw muscle spindle afferent projection in the cat was "relatively weak." This suggestion was based on their finding that EPSP's could be recorded in only a small fraction of jaw-closing motoneurons using the technique of spike-triggered-averaging from single afferents in the mesencephalic nucleus of the fifth nerve. Appenteng et al. suggested that this relatively selective projection might be appropriate for the jaw-closing muscles which are complex in their anatomy and functions. In other words, spindles from one part of a muscle would project only to those motoneurons with mechanically similar actions. This interpretation is consistent with the views of Botterman et al. (Amer. Zool., 18: 135-152, 1978) that afferents of most muscle systems are organized in terms of muscle compartments.

When a stretch reflex is elicited by displacing the mandible, all of the jaw-closing muscles are stretched, presumably synchronously, producing a large, synchronous discharge of muscle spindle afferents into the motoneuron pool. In pilot experiments we found that mechanical indentation of the tissue overlying jaw-closing muscles reliably elicited jaw stretch reflexes. Use of this stimulus allows more restricted activation of spindles and can be used to study the distribution of the stretch reflex in humans. A systematic study of responses of ten normal subjects was undertaken to determine the distribution of the responses to this "localized" stretch stimulus across synergistic muscles and across regions within one muscle. Our results show that the reflex response to this stimulus is not confined to the muscle stimulated, rather it is distributed across all jaw-closing synergists. In addition, there was no evidence that the "on-site" reflex response (response at the site of stimulation) as measured from the EMG tended to be larger than the "off-site" response. Our data support the hypothesis that the jaw stretch reflex pathway in humans is potent but do not support the idea of rigid compartmentalization of this reflex pathway.

PAIN: AFFERENT NOCICEPTORS

229.1 A QUANTITATIVE, ITCH-PRODUCING STIMULUS. R.P. Tuckett, Department of Physiology, University of Utah, Med. Ctr., Salt Lake City, Utah 84108.

This report focuses on two issues that are fundamental to the study of experimental pruritus. First, investigation of the sensation of itch has had a disdvantage not shared with other cutaneous modalities in that there has been no reproducible technique for inducing pruritus. A needle (20 guage) was attached to a strain guage transducer to aid in reproducibly penetrating the skin of human subjects who had given prior informed consent. Papain was chosed as a pruritogenic agent because of its ability to cause itching without mast cell degranulation. (Progressive dilutions of papain were prepared, starting from near saturation (150, 75, 37, 15, 7 and 0 units/ml, Sigma, St Louis, MO) in Hepes buffer, ph=7.4, and Cleland's reagent (a reducing agent to maintain papain reactivity, Sigma). Sterility was maintained by using a .22 um disk filter and sterile containers and was checked periodically by incubating samples in culture media and looking for bacterial growth. Solutions were applied in pairs to the forearms of human subjects, double blind and in pseudorandom order, with one member remaining the same (37 units/ml) while the other was varied.

The second issue to be addressed is that unlike other cutaneous modalities, my knowledge no experiments have been done relating the intensity of pruritic sensation to stimulus strength. A cross-modality psychophysical experiment was performed in which subjects were ask to match the intensity of itch with the intensity of a light, for the stimulus pairs described above. The subject controlled the voltage supplied to the light by turning a knob. The voltage waveform was digitized and analyzed by obtaining measures of itch magnitude, latency, time to peak, peak amplitude and duration. A value for itch magnitude was obtained by integrating the amount of voltage applied to the light over a standard period of time (3 min from the start of pruritus). Although no correlation (Kendall correlation coef.) was found between the absolute values of these variables and stimulus strength, there was significant positive correlation between the ratio of paired values and stimulus strength for itch magnitude (N=39, $p<0.0001$), time to peak (N=22, $p<0.0001$), peak amplitude (N=23, $p<0.02$) and duration (N=9, $p<0.08$), suggesting that day-to-day variation in human perception has a significant impact on the sensation of itch. No correlation was found between the ratio of latencies and papain concentration.

229.3 CHEMICAL SYMPATHECTOMY MAGNIFIES CAPSAICIN-INDUCED DEFICITS IN SENSATION AND NEUROGENIC INFLAMMATION: PARALLELS TO DYSAUTONOMIA. T. J. Coderre*, F. V. Abbott and R. Melzack*. Dept. of Psychology, McGill Univ., Montreal, P.Q. H3A 1B1

Pain sensitivity is mildly depressed by both capsaicin and chemical sympathectomy. This study reports on the effects of combining these two treatments on pain, tactile sensitivity and inflammation. One month after application of 1.5% capsaicin (CAP) to the sciatic nerve, rats were treated with 30 mg/kg of guanethidine (GUA) for 4-6 days. The effects of the combined treatment or each drug alone were as follows. Heat pain: The latency to withdraw the hind paw from 50°C water was increased by 47% by GUA, 240% by CAP, and 410% by CAP + GUA. Cold pain: Foot-withdrawal latencies from -6°C brine were unaffected by any of the treatments. Inflammatory pain (Formalin test): Behavioural responses to a s.c. injection of 0.05 ml of 2.5% formalin into the plantar surface of the hind paw were reduced by 60% by GUA, not at all by CAP, and 74% by CAP + GUA. Inflammatory response: Leakage of Evans Blue dye (50 mg/kg i.v.) was used as an index of neurogenic inflammation. After s.c. injection of 0.05 ml of 1.0% formalin into the plantar surface of the hind paw, the leakage of dye into the injected paw was mildly inhibited by GUA. Both CAP and CAP + GUA produced a marked reduction of the dye leakage. CAP inhibited dye leakage for 30 min., while CAP + GUA's effects lasted 3-4 hrs. Tactile sensitivity: Neither GUA nor CAP affected the latency for rats to detect and remove 8 mm discs of sticky tape applied to the dorsal and plantar surfaces of the hind paw. CAP + GUA produced a marked deficit in this test.

Thus, combining capsaicin with an antisympathetic treatment has a powerful synergistic effect on heat pain, inflammatory pain, tactile sensitivity and inflammation. The effects imply that these treatments interact to produce a large decrease in both the sensitivity of cutaneous receptors and the vegetative functions involved in responses to injury. In light of these data, it is interesting that the neuropathology of familial dysautonomia involves both peripheral sympathetic denervation and a preferential loss of unmyelinated afferents. The present data suggest that the mechanisms underlying the reduction in cutaneous sensitivity and neurogenic inflammatory responses in dysautonomia may involve an interaction between the peripheral sympathetic system and peptidergic afferents.

229.4 EVIDENCE FOR EPHAPTIC CONDUCTION BETWEEN UNMYELINATED FIBERS IN THE PERIPHERAL NERVE OF PRIMATES R. A. Meyer, S. N. Raja, J. N. Campbell and R. Burke*. Applied Physics Lab. and Depts. Neurosurgery and Anesthesiol./CCM, Johns Hopkins U., Balto., MD 21205

Coupling of electrical activity between peripheral nerve fibers, presumably ephaptic conduction, has previously been observed following various forms of nerve injury. Recently, coupling between myelinated nerve fibers in normal nerves of cats has been reported. We found that coupling occurs between unmyelinated, but not myelinated,nerve fibers in the normal peripheral nerve of the primate. In several cases, the nerve fibers were connected to a cutaneous nociceptor. Primates (3 baboons and 6 monkeys) were anesthetized with either pentobarbital infusion (4-6 mg/kg/hr) or halothane/N_2O (1.0%/66%). The animals were paralyzed with pancuronium and mechanically ventilated to eliminate artifacts due to muscle twitching. Recordings from single fibers in the superficial radial nerve were obtained from fine teased nerve strands. The strands were cut proximally such that only centripetally directed action potentials were recorded. Stimulating electrodes were placed on the parent nerve 3 cm proximal (PSE) and 3 cm distal (DSE) to the recording site. Action potentials at the recording site evoked by PSE stimulation were considered as an indication of coupling between nerve fibers. With stimulus intensities at the PSE low enough such that only A-beta and A-delta fibers were activated, no action potentials were recorded. However, in 20 cases, PSE stimulation intensities sufficient to excite unmyelinated fibers resulted in action potentials at a fixed delay at the recording electrode. Simultaneous stimulation of the PSE and DSE resulted in collision of this action potential only at DSE intensities sufficient to excite C-fibers. Collision techniques were also used to demonstrate the bidirectionality of this coupling. The conduction delays and the requirement for high intensity stimulation were indications that the coupling was between unmyelinated fibers. In at least 4 cases, one of the fibers involved in the coupling was a cutaneous nociceptor. Based on conduction delays and procaine application to the receptive field, the site of the coupling was estimated to be near the receptor. The time interval between stimulation at the PSE and arrival of the action potential at the recording site was transiently increased by mechanical stimulation of the receptive field. In one case tested, stimulation at the PSE (10 Hz for 3 sec) resulted in a transient reduction in response to heat at the receptive field of the nociceptive afferent. A likely explanation for these observations is that ephaptic transmission occurs between unmyelinated fibers in the normal peripheral nerve of primates. (Support: DOD contract #N00024-83-C-5301 and NIH grants NS-14447 and NS-00519)

229.5 SENSITIZATION OF UNMYELINATED NOCICEPTIVE AFFERENTS BY HALOTHANE, S.N. Raja, R.A. Meyer, J.N. Campbell, and R. Burke* The Johns Hopkins University, Baltimore, MD 21205

We recently reported that the response of nociceptive primary afferents to heat stimuli was altered by different general anesthetics. This study was designed to determine the following: Is the response raised by halothane or lowered by barbiturates? Is the anesthetic effect due to direct action on the cutaneous receptor or an indirect effect? The response to heat stimuli of C-fibers sensitive to both mechanical and heat stimuli (CMHs) were studied in monkeys. All fibers studied innervated the glabrous skin of the hand. The animals were mechanically ventilated and end-tidal CO_2 maintained between 35-40 torr. Heat stimuli were produced by a laser thermal stimulator. To determine the effects of halothane, monkeys were anesthetized with pentobarbital (IV infusion of 4-6 mg/kg/hr) and the responses to repeated heat stimuli of 5 CMHs were recorded while the inspired concentration of halothane was manipulated. The response of the fibers increased monotonically as the halothane concentration increased and returned to baseline when halothane was stopped. The mean response at 2% halothane was 191 $\pm$ 8% (S.E.M.) of the response without halothane. Comparable experiments were done using IV infusions of 1-4 mg/kg of methohexital with the monkeys anesthetized under 1% halothane and 66% nitrous oxide. The CMHs showed only a small if any change in response. We conclude that the difference in response with different anesthetics is mainly due to an increase in response induced by halothane. To see if the change in blood pressure induced by halothane might be responsible for the change in CMH response, blood pressure was maintained above the baseline using phenylephrine or ephedrine. These vasopressors by themselves induced no change in CMH response, whereas the response was still increased by halothane. In two experiments the the skin temperature was monitored as an index of skin perfusion. Following 10 min. of 1.5% halothane, the response of the CMHs to heat stimuli increased significantly whereas the skin temperature changed minimally. In additional experiments, sympathetic activity was blocked by procaine applied to the nerve proximal to the recording site or by section of the brachial plexus at the root level. Again, the response of the C-fibers increased when halothane was administered. In summary the response to heat of C-fiber nociceptive afferents increased in a dose-dependent manner to halothane. Alterations of blood pressure, skin perfusion, sympathetic tone, core temperature, or end-tidal CO_2 do not account for the observed increased response. The most likely explanation is that halothane sensitizes reversibly the nociceptive receptor in the skin. (N.I.H. grants NS-14447 and NS-00519)

229.6 IDENTIFICATION OF CUTANEOUS FIBRE GROUPS RESPONSIBLE FOR SIGNALLING NOXIOUS HEAT AND NOXIOUS MECHANICAL STIMULI IN THE RAT R. Doucette, E. Theriault and J. Diamond. Dept. of Neurosciences, McMaster University, Hamilton, Ontario L8N 3Z5.

When the back skin of a rat is lightly pinched or heated it activates the cutaneous endings of nociceptive fibres that run in the dorsal cutaneous nerves (DCNs) and reflexly excites the underlying cutaneous trunci muscle (CTM). Direct electrical excitation of the DCNs has revealed that only the Aδ and C fibres elicit reflex responses in this muscle, and that these are of clearly different latencies (15-20 msec and 60-80 msec respectively). We wanted to find out which fibre groups are activated by which particular nociceptive modality. To answer this question we used capsaicin, which is toxic to C fibres, but affects Aδ fibres minimally. In order to compare our results with those of others we used several additional behavioral tests; these included the tail flick reflex and the withdrawal response of paws to noxious mechanical and thermal stimuli. Two to three day old female Wistar rats were given a single subcutaneous injection at the back of the neck of either capsaicin (50 mg/kg) or vehicle; the tests were conducted 2-5 months later. The performance of vehicle-treated rats did not differ from untreated controls. Both the C fibre and the heat evoked component of the CTM reflex were absent in all capsaicin treated rats, but there was no detectable alteration in either the pinch or the Aδ component of this reflex. In accord with these results, noxious heat applied to either the hind paw or the tail evoked no response, or one with a significantly longer latency, but the animals still exhibited a normal reflex withdrawal to a pinch of the front and hind paws. However, though the skin behind the pads of the hind paws of the capsaicin-treated animals was relatively insensitive to thermal stimuli, that on the pads was only slightly less sensitive than normal. Similarly the heat sensitivity of the pads of the front paws seemed unaffected by the capsaicin even when it had been injected directly into the pad. One explanation of these findings is that noxious heat on the paw pads is not signalled by C fibres. We examined this possibility by using intravenously administered Evans blue dye to study the extravasation of plasma proteins that occurs when the peripheral terminals of nociceptive C fibres are antidromically excited. In normal, untreated animals extravasation was seen in all skin regions except in the pads of both front and hind paws. We conclude that (a) thermal nociception in skin regions other than paw pads is signalled primarily by C fibres, but in the pads most likely by Aδ fibres, and (b) noxious mechanical stimuli in all skin regions, including the paw pads, activate Aδ fibres.

(Supported by Multiple Sclerosis Society of Canada).

229.7 A COMPARISON OF TRIGEMINAL THERMORECEPTORS OBSERVED IN CATS AND IN MONKEYS. D.A. Poulos and H. Hirata, Dept. of Anatomy and Div. of Neurosurgery, Albany Medical College, Albany, NY 12208.

In examining details of the response characteristics of primary afferent trigeminal cold receptors (T units), certain differences were observed between cat and monkey neurons. Extracellular recordings were obtained from trigeminal ganglion neurons in anesthetized (Nembutal and/or urethane) cats and in two species of monkeys (Saimiri sciureus and Macaca mulatta), the latter showing no differences between their T unit responses. Thermoreceptive neurons were tested using a standard sequence of skin temperatures held constant and step-like cooling stimuli below a 35°C preadapting standard.

All T units maintained temperature-dependent discharge activity over a range of constant skin temperatures. The steady temperature response profiles approximated bell-shaped distributions, with peak discharge rates for both types of animal occurring at 29°C. Cat T units, however, had decreased average firing rates e.g. 8/sec at 29°C as opposed to 15/sec in monkeys. Conversely, cat T units were responsive over a wider range of constant temperatures (45-9°C in cats, as opposed to 41-17°C in monkeys). In addition, bursting patterns of steady-state discharge activity were commonly seen in monkey T units while they were rarely observed in the cat.

The dynamic response profiles of T units to a series of rapid cooling stimuli starting from a 35°C preadapting temperature were similar for the two types of animal with the following exceptions: firstly, cat T units appeared to be more sensitive to smaller cooling steps since maximal dynamic activity was obtained within 2-4° cooling, whereas the averaged dynamic responses of monkey T units required 8° cooling to reach maximum firing rates. Secondly, over the colder range of stimulus temperatures (19°C and below) monkey T unit dynamic responses were brief and invariably followed by a post-excitatory cessation of activity for the remainder of the stimulus period. Cat T units, by comparison, did not display post-excitatory cessation over the range of temperatures tested, although a brief pause or slowing of firing sometimes occurred followed by a recovery of discharge. The latter result is consistent with the finding that cat T units are active over a much colder range of constant skin temperatures than are monkey T units.

Since the experimental methods used in these studies were identical, the observed differences in the behavior of cat and monkey thermoreceptors can thus far only be assigned to a "species" difference.

Supported by NIH Grant NS 11384.

229.8 PROPERTIES OF CELL BODIES INNERVATING THE PULP CHAMBER OF PRIMARY AND PERMANENT CANINE TEETH. Terrell E. Jones*, Kenneth V. Anderson, and Norman F. Capra. Department of Anatomy, University of Mississippi Medical Center, Jackson, MS.

Many studies concerned with the innervation of tooth pulps have focused on the properties of axons near the point of entry to the pulp chamber. In general, studies of this type have characterized axons with regard to their degree of myelination, diameter and whether the fibers were afferent or efferent in function. The present study was designed to systematically examine the morphological properties of the cell somata of neurons in the trigeminal ganglia (TG) that innervate the canine teeth in cats. Special attention was focused on comparisons between neurons that supply primary teeth in kittens and those that supply permanent teeth in adults.

To identify TG neurons supplying canine teeth, horseradish peroxidase (HRP) was injected into the left upper canine teeth of four five-week-old kittens and four adult cats. Multiple, unilateral injections of HRP (Sigma VI, 40% solution) were made into the pulp chamber of each cat. Animals were euthanized and transcardially perfused 48 hours after the final pulpal injection. Frozen, serial sections (25 micra) were made of each TG and the tissue processed using the TMB method to enable visualization of the HRP reaction product.

Labeled cells were identified in each animal using bright field optics. A total of 713 labeled cells were observed in the four kittens, for an average of 178 TG neurons supplying each canine tooth in kittens. By contrast, 704 labeled neurons were observed in the adult cats for an average of 176 neurons supplying each canine tooth. The average diameters and perimeters of cell bodies supplying kitten canine teeth were 45.13 microns and 135.12 microns, respectively, while the average areas measured 1180.72 microns^2. In the case of neurons supplying permanent canine teeth in adult cats, the average diameters, perimeters and areas were 54.16 microns, 159.38 microns and 1473.82 microns^2, respectively.

The results of our studies indicate that single canine teeth in kittens are supplied by about the same number of TG neurons as are the canine teeth in adults. This is a somewhat surprising result, since the TG neurons have to supply greater numbers of adult teeth than kitten teeth (28 vs 20). There are, however, significant differences in the sizes of TG neurons that supply the teeth in kittens and adults, with those in kittens being generally smaller than those in adults. Studies are presently underway to identify the factors that might account for the differences in cell sizes observed in the present investigation and to functionally characterize the TG cells that supply the canine teeth in kittens.

229.9 TRIGEMINAL AXON COLLATERALS FROM SINGLE CELL BODIES DO NOT EXPLAIN REFERRED HEAD PAIN. T. O'Connor* and D. van der Kooy (Spon: A. Jakubovic). Department of Anatomy, University of Toronto, Toronto, Canada M5S 1A8.

The trigeminal nerve provides the main sensory pathway involved with intracranial head pain. Recent studies with cats have shown that afferent fibers from both the forehead and the middle cerebral artery have cell bodies in the same region of the opthalmic branch of the trigeminal ganglion, thus suggesting the possibility of bifurcated axons as a mechanism for referred head pain.

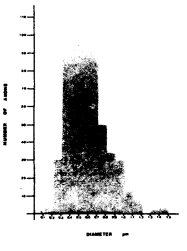
We have found that afferent trigeminal fibers to the forehead region of the rat, (frontal branch of the opthalmic division), do not share the same cell bodies as fibers to the middle cerebral artery. We have used a retrograde fluorescent double labelling technique in these experiments. True blue (5%, .2ul) was injected around the proximal region of the middle cerebral artery. The frontal branch of the opthalmic division of the trigeminal nerve was dissected out 1 day later and the proximal end was soaked in nuclear yellow (1%). Two days after the nuclear yellow injection the rats were anesthetized and sacrificed by intracardiac saline and then formalin perfusion. Ganglia were sectioned in a cryostat (32 um) and were examined with a heitz epifluorescent microscope.

Labelled cell bodies were distributed primarily in the opthalmic branch of the trigeminal ganglion. The labelled cells bodies were labelled with either true blue (blue fluorescing cytoplasm) or nuclear yellow (yellow fluorescing nucleus). There were no double labelled cells. In the opthalmic division most of the true blue labelled cells from the middle cerebral artery injections were nearby nuclear yellow labelled cells from the frontal branch injection. Thus it seems unlikely that trigeminal ganglion cells with bifurcating axons can provide a full explanation of referred headache pain. A more central mechanism would seem to be called for, however an interaction between the processes of cell bodies in the trigeminal sensory ganglia remains a possibility.

229.10 DIAMETERS OF AFFERENT UNMYELINATED AXONS IN THE CAT ARTICULAR NERVES. L.A. Langford. Physiologisches Institut der Universität Würzburg. D-8700 Würzburg. Federal Republic of Germany.

The medial and posterior articular nerves supplying the cat knee joint are considered to consist mainly of myelinated axons with only a small number of unmyelinated axons which are thought to be postganglionic efferent (sympathetic);however, Langford and Schmidt (Anat. Rec. in press, 1983) have demonstrated that 80% (800) of the total axons in the medial articular nerve are unmyelinated and only 20% (200) are myelinated. Similarly, the posterior articular nerve is 78% unmyelinated. Of the total axons in both nerves, all myelinated and 50% of the unmyelinated axons have their origins in the dorsal root ganglion cell and are therefore sensory. The remaining 50% of the unmyelinated axons arise from the sympathetic chain. In the present study the diameter distribution of this significant number of afferent unmyelinated axons is being determined in order to further characterize the most fundamental aspects of joint innervation.

After bilateral sympathectomy (L_3-S_3) and a degeneration time of 10-12 days, the animals were perfused with a 3% glutaraldehyde-3% paraformaldehyde solution. Sections of the medial and posterior articular nerves were removed and prepared for EM analysis according to the method of Langford and Coggeshall (Anat. Rec. 197: 297-303,1980). Montages of the nerves in cross section were made at a magnification of 10,000. The diameters of all unmyelinated axons were measured. The preliminary results from the medial articular nerve are shown in the following histogram.



Diameter measurements of the unmyelinated axons in the posterior articular nerve are in progress. Diameters of postganglionic efferent unmyelinated axons in deafferented articular nerves will also be compared to the afferent population.

Supported by the Alexander von Humboldt Foundation, Fed. Rep. of Germany.

229.11 AN ELECTRONMICROSCOPIC ANALYSIS OF THE INFRAORBITAL NERVE IN RAT. R.D. Mooney, A. Hess, M. F. Jacquin and R.W. Rhoades. (SPON: G. Krauthamer) UMDNJ-NJSOM & RMS, Piscataway, NJ 08854

The central representation of the mystacial vibrissae in rodents has become an important model for studying peripheral influences upon normal central nervous system development and also the effects of peripheral injury on central neural ontogeny. The infraorbital (IO) branch of the maxillary division of the trigeminal nerve innervates the vibrissae and, surprisingly in view of its importance as a model system, little is known regarding the normal composition of this nerve or the manner in which its organization might be altered by neonatal damage. As a first step toward answering these questions we have described the normal organization of the IO nerve using electronmicroscopic methods. Four nerves have been examined thus far. Just caudal to the infraorbital foramen at the level anterior superior alveolar foramen the nerve is composed of 18 to 25 bundles which range from 575 to 87,923 μm^2 in cross sectional area. Axon counts from thin sections taken at this level (all myelinated and unmyelinated axons were counted in each nerve) demonstrated that the IO nerve contains an average of 19,740 myelinated (s.d.=2,054) and 13,319 unmyelinated (s.d.=1,159) fibers. The maximum number of myelinated axons in a single bundle was 3,068 (s.d.=821) and the maximum number of unmyelinated axons was 2,464 (s.d.= 1,220). The largest number of myelinated and unmyelinated axons were generally found in the same fiber bundle.

All myelinated and unmyelinated axons along the major and minor axes of each bundle in each nerve were measured. Areas (including myelin sheaths for medullated fibers) were computed with a graphics tablet and converted to standard diameters. The average for the myelinated axons (N=5,207) was 4.42 μm (s.d. =1.76) and the fiber diameter distribution was essentially unimodal. That for the unmyelinated axons (N=1,052) was 0.60 μm (s.d.=0.16) and the distribution was again unimodal.

We have thus far examined one IO nerve from an adult rat subjected to section of this V branch at birth. The nerve (all data are from sections taken at the same level as those for the normal nerves) was comprised of 20 bundles with cross-sectional areas of 284-30,352 μm^2. It contained 5,260 myelinated and 2,747 unmyelinated fibers. The average diameter for the myelinated axons (N=472) was 3.01 μm (s.d.=1.1) and that for the unmyelinated fibers (N=175) was 0.61 μm (s.d.=0.20).

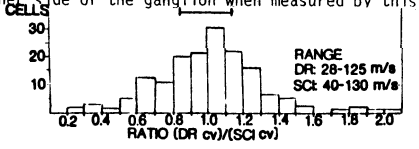
Supported by DE06528, EY04710, EY03546, The March of Dimes and the UMDNJ Foundation (RWR).

229.12 CONDUCTION VELOCITY CHANGES ALONG LUMBAR PRIMARY AFFERENT FIBERS IN CATS. A.J. Rindos and G.E. Loeb, Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

Microelectrodes were inserted into L7 dorsal root ganglia (DRG) to record spikes from primary afferents. These were used to trigger simultaneous averaged records of activity from multiple tripolar electrode hooks around the L7 dorsal root (DR) and nerve cuffs around the sciatic (SCI) and posterior tibial (PT) nerves. Latencies were used to determine conduction velocities (cv) between various tripolar electrode pairs. A histogram of the ratios of overall DR to SCI cv is plotted below. The bar above the graph indicates an estimate of the range of uncertainties due to hardware limitations. Most units exhibited no significant change between SCI and DR cv, but PT cv averaged 85% of SCI cv. Changes in local SCI cv did not exceed experimental error. A current amplitude calibration pulse indicated no correlation between changes in local cv's and action current amplitudes.

CV's were also obtained by stimulating SCI or DR electrodes and measuring arrival time of the unit spike in the DRG. Distances were measured by laying a string from DRG center to stimulating cathode. SCI cv's obtained by stimulation and averaging were comparable for a given unit. However, DR cv's obtained by stimulation were consistently slower than those obtained by averaging. This underestimate was probably due to the relatively larger effect of the conduction delay between bifurcation and soma in the DRG. This variable delay also affected cv measured by latency between DRG spike and a single averaged potential.

Spike-triggered averaging between adjacent tripolar electrodes appears to be the most accurate way to measure cv, but it does have two significant sources of error: 1) For short latencies, digital sampling bin widtn introduces a significant uncertainty in peak time; for long latencies, sampling rates must be reduced to allow sufficient pretrigger storage in some averagers. Increased separation of electrodes reduces this problem. 2) Tripolar electrodes at the edge of an array may produce monopolar-like recordings with distorted waveforms which shift the observed peak. Nerve damage is also most likely at the edges due to kinking. CV in DRG axons was not significantly different on either side of the ganglion when measured by this technique.



229.PO THE ROLE OF ENDONEURIAL K^+ IN PROMOTING SPONTANEOUS ACTIVITY IN REGENERATING MAMMALIAN NERVE - SINGLE FIBER AND ENDONEURIAL K^+ RECORDINGS, P.A. Low. Neurophysiology Laboratory, Mayo Clinic, Rochester, MN 55905.

While extracellular K^+ is known to affect peripheral nerve membrane potential and excitability, its role in modulating spontaneous activity (SA) in damaged nerve fibers is largely unexplored.

The $[K^+]_e$ of normal rat peripheral nerve (sciatic and tibial) has recently been studied and found to be 3.7 mM (sd 0.7 mM)[1]. The present report is confined to the endoneurial K^+ and its relationship to spontaneous activity of regenerating nerve fibers. The left tibial nerve was crushed in 22 Sprague-Dawley rats and the endoneurial K^+ measured using K^+-ion sensitive microelectrodes 2 days to 3 months following crush. The $[K^+]_e$ was increased in the first 2 weeks with a progressive decline towards control values after that. Single fiber recordings were made from the tibial division of sciatic nerve in 13 animals. The mean number of single fibers studied per rat was 55.5 (sd 24.6). Spontaneous activity was present in all regenerating nerves (7-50% of single fibers had SA) and was maximal in the first 2 months (especially 2-4 weeks). Endoneurial K^+ was further increased by intra-arterial injection of potassium rich injectate into the supplying saphenous artery. Spontaneous activity was markedly enhanced when $[K^+]_e$ began to rise; there were further superimposed bursts of SA with the bolus. These findings suggest that $[K^+]_e$ increase is factor in enhancing SA in damaged nerve fibers.

PAIN: CENTRAL PATHWAYS

230.1 EFFECTS OF TRIGEMINAL TRACTOTOMY ON THE RAT'S PERCEPTION OF NOXIOUS AND NON-NOXIOUS FACIAL STIMULI. J. G. Broton and J. Peter Rosenfeld. Department of Psychology, Northwestern University, Evanston, IL 60201.

Trigeminal (V) tractotomy is a surgical procedure whereby the caudal V nuclear complex is selectively deafferented. When performed in cases of V neuralgia, patients are often relieved of the facial pain spasms, and become analgesic to noxious stimuli applied to the face. Perception of non-noxious stimuli remains. Primarily because of this evidence, the caudal V nuclear complex (nucleus caudalis) has often been considered to be the only part of the V complex which is important for the normal perception of facial pain. This belief has influenced animal researchers studying the mechanisms of facial nociception, such that almost all are directed to nucleus caudalis. Recent studies, however, have found that tractotomy does not result in dental analgesia in cats, monkeys or trigeminal neuralgia patients, nor in perioral facial analgesia in monkeys. We here report that rats receiving tractotomy-like knife cuts do not exhibit deficits in responsiveness to noxious heat delivered to several areas of the face.

Adult male Holzman albino rats were used. During testing, rats were tail-restrained and a heater device was attached to a previously implanted socket on the rat's head. Noxious heat stimuli were applied to each of five sites on the face, resulting in the production of nocifensive face-rubbing responses (FRs). Four days of baseline testing assured the reliability of responses. Tractotomy-like coronal cuts were made with a custom-made wire knife, which was lowered through a chronically implanted guide cannula. The effectiveness of the cuts was assessed by viewing the Fink-Heimer-stained degeneration in the descending V tract caudal to the cut. Rats were retested 24 hours after the cuts.

Post-tractotomy behavioral data on nine rats have thus far been collected. Results indicate that V tractotomies often did not produce significant elevations in FR latencies at any of the five facial sites tested. In three rats, increases were seen at perioral positions, while at non-perioral sites, FR latencies were at control levels. Reasons for the individual cut differences will be discussed. Work in progress will assess the effects of similar knife cuts on the perception of non-noxious air puff and heat stimuli, in addition to noxious heat. (Supported by NIH Grants GM23696 and DE05204 to JPR.)

230.2 RESPONSES IN TRIGEMINAL SENSORY NUCLEI TO TOOTH-PULP STIMULATION IN THE CAT:EFFECTS OF SURGICAL TRAUMA. R.W.Clarke* and B.Matthews. Dept. of Physiology, University Walk, Bristol BS8 1TD, U.K.

To excite trigeminal brain-stem neurones by electrical stimulation of tooth-pulp in an acutely-prepared cat in a stereotaxic frame, often requires stimulus intensities which are greatly in excess of those needed to excite the majority of the myelinated pulpal fibres, yet the latencies of the responses indicate that they are due to myelinated afferents. Furthermore, although approximately 40% of primary afferents from tooth-pulp respond to thermal stimulation of the intact tooth (Kollmann,W.,Matthews,B. & Suda,H., J.Physiol, 332 63P, 1982), attempts in our laboratory to record responses of neurones in the brain-stem to such stimuli have been unsuccessful. Other observations indicated that injury created in setting up the acute preparations caused a profound depression in the excitability of trigeminal neurones, lasting several hours. We have therefore investigated brain-stem responses to electrical and thermal stimulation of teeth in animals in which trauma immediately prior to recording was kept to a minimum.

In nine cats, a preliminary operation was carried out in which a small chamber was attached to the skull so that the head could be fixed and recordings made with minimal trauma on a subsequent occasion. A further three animals were prepared acutely with precautions to minimise trauma, including the use of atraumatic ear-bars. Ten other cats were prepared normally, without these precautions. Recordings were made using glass-coated tungsten microelectrodes and recording sites marked by small electrolytic lesions. All procedures were carried out under alphaxalone/alphadolone anaesthesia. Data were collected only when arterial blood pressure and end tidal CO_2 were within normal limits.

In both groups of animals prepared with minimal trauma, more than 95% of the units which responded to electrical stimulation required only a single stimulus of less than 100μA, 0.1 ms to excite them, comparable with the thresholds of the primary afferents. In cats prepared in the standard way,the thresholds of only 30% of units were in this range and 30% were above 1mA,0.1ms. There were no differencies in the latencies of the responses between groups. Of 197 units tested with thermal stimuli in minimally-traumatised cats, 101 responded preferentially to cooling (10^oC)of the intact tooth and 9 responded preferentially to heating (50^oC). Units were found at all levels of the trigeminal nuclei. These results show that greater summation of inputs is required to activate trigeminal neurones from tooth pulp in cats prepared in the standard way than if trauma is minimised. The mechanism of the depression has not been established. Supported by M.R.C.

230.3 CONVERGENT AFFERENT INPUTS AND RESPONSES EVOKED BY NOXIOUS AND NON-NOXIOUS OROFACIAL STIMULI OF NEURONES IN TRIGEMINAL (V) SUBNUCLEUS ORALIS. J.W. Hu*, G. Zhong*, N. Amano* and B.J. Sessle (SPON: F. Coceani). Fac. of Dentistry, University of Toronto, Toronto, Canada M5G 1G6.

Since subnucleus oralis as well as subnucleus caudalis (medullary dorsal horn) of the V spinal tract nucleus has been recently implicated in V brainstem mechanisms of pain, we have examined the response properties of oralis neurones to a variety of natural and electrical stimuli. Extracellular activity was recorded from 250 single oralis neurones in chloralose-anaesthetized cats; each neurone was functionally classified on the basis of its cutaneous receptive field properties as low-threshold mechanoreceptive (LTM), wide dynamic range (WDR), or nociceptive-specific (NS). In addition to noxious (e.g. pinch, heat) and non-noxious (tactile) stimuli, each neurone was tested for responsiveness to electrical stimulation of various cutaneous and oral mucosal sites, canine and premolar tooth pulp, visceral (superior laryngeal nerve) and C_3 neck afferents, and hypoglossal and temporalis muscle afferents; recording sites were confirmed histologically.

We verified previous findings of the "inverted head" somatotopy of oralis. The receptive field of 50% of the neurones was localized to the facial skin; for 25% it was restricted to the oral cavity, and for the remaining 25% it involved both cutaneous and intraoral sites. LTM neurones having localized orofacial receptive fields comprised 90% of the sample. The remaining neurones located within oralis were WDR and NS neurones; many nociceptive neurones were also located outside oralis in the adjacent reticular formation. The oralis nociceptive neurones responded to pinch, and some also responded to noxious radiant heat or algesic chemicals injected into lingual arterial branches supplying the tongue muscles. In terms of electrically induced responses, 50% of the oralis neurones could be excited by cutaneous or oral mucosal electrical stimuli applied outside the V division of their receptive field. Furthermore, 30% were excited by canine and 35% by premolar pulp stimulation. Visceral, neck, hypoglossal and temporalis afferent stimulation excited 35%, 10%, 20%, and 35%, respectively. The incidence of excitation from these convergent afferent inputs was higher for the WDR and NS neurones than the LTM neurones.

This study has confirmed that oralis is a somatotopically organized structure predominantly composed of LTM neurones. A small proportion of nociceptive neurones does however occur within oralis. Convergent afferent inputs evoked by electrical stimuli are prominent, and are particularly apparent in the nociceptive neurones.

Supported by NIH grant DE04786.

230.4 TRIGEMINAL MEDULLARY DORSAL HORN NEURONS PROJECTING TO MEDIAL AND LATERAL THALAMUS. Carmella Angerbauer and Jonathan O. Dostrovsky (SPON: H.C. Kwan), Department of Physiology, University of Toronto, Toronto, Canada, M5S 1A8

The caudal segment of the trigeminal spinal nucleus, termed subnucleus caudalis or the medullary dorsal horn (MDH) is functionally and anatomically similar to the spinal cord dorsal horn and is believed to be the major relay site for nociceptive sensory input from the oral-facial region. The present study examined the characteristics of MDH neurons projecting to medial and/or lateral regions of thalamus.

Experiments were performed on 28 cats anesthetized with chloralose. Single unit extracellular recordings of MDH neurons were obtained using standard methods. An array of bipolar stimulating electrodes was placed in the contralateral thalamus. All stimulation sites were determined from cresyl violet stained histological sections. Neurons fulfilling the criteria for antidromic excitation from thalamus were characterized using various innocuous and noxious mechanical and thermal stimuli. A total of 29 projection neurons were examined in detail, of which 12 could be antidromically excited by stimulation in medial thalamic regions (parafascicular, subparafascicular, central medial, and principal ventromedial nuclei), 12 by stimulation in lateral thalamic regions (ventrobasal complex, zona incerta, and posterior complex) and 5 by stimulation in both regions. Neurons excited by stimulation in medial sites consisted of 6 nociceptive specific, 5 low threshold mechanoreceptive, and 1 innocuous thermal (cold). Neurons excited by stimulation in lateral thalamus consisted of 7 nociceptive specific, 2 wide dynamic range, and 3 low threshold mechanoreceptive. Those projecting to both regions were all low threshold mechanoreceptive. 4 neurons had receptive fields spanning the whole ipsilateral face, 3 had fields extending bilaterally across the whole body, and the remainder had relatively small fields within the trigeminal region. There was no clear relationship between receptive field size and thalamic stimulation site. These results indicate that in addition to the previously reported trigeminal projection to ventroposteromedial thalamus, there is a significant projection to more medial sites in thalamus.

Supported by NIH grant DE-05404.

230.5 PROPERTIES OF SPINOTHALAMIC TRACT CELLS LYING IN THE VENTROMEDIAL ZONE OF THE LUMBAR SPINAL CORD IN THE RAT. D. Menétrey, J. de Pommery (SPON:E.R. PERL). INSERM, Unité de Neurophysiologie Pharmacologique, U 161, 2 rue d'Alésia, 75014 Paris, France.

In an earlier study using the retrograde transport of horseradish peroxidase (Giesler et al., J. Comp. Neurol.,184 : 107, 1979) we have shown that a prominent spinothalamic projection issues from the ventromedial zone (VMZ) of the lumbar dorsal horn of the rat's spinal cord. A similar finding has been reported in the cat but not in the monkey (Trevino and Carstens, Brain Res., 98 : 177, 1975). Following confirmation of this projection in the rat (Chaouch et al., J. Comp. Neurol., 214 : 309, 1983) we undertook to characterise electrophysiologically it by studying antidromically identified spinothalamic tract (STT) neurons in immobilized rats under halothane anesthesia. Extracellular recording sites were located by injection of a dye, VMZ sites were selected from camera lucida observations.

Lumbar VMZ cells activated from the posterior levels of the thalamus had conduction velocities ranging from 11 to 27 m/s (m=18 m/s). As was predictable from their densely packed formation, the cell population of VMZ was very homogeneous in terms of its electrophysiological properties. They were activated from peripheral areas including both proprioceptive and cutaneous tissues. Effective stimuli were combinations of the joints and skin. Their background activities were directly related to the resting position of joints. A) The most frequent subpopulation had restricted ipsilateral receptive fields including ankle, joint of the digits and skin. Their background activity consisted of small groups of spikes (mostly doublets and triplets) which depended on the resting position of the ankle (flexion reduced it while extension increased it). The resting position of the ankle also potently affected the excitatory responses from skin (touch, pressure) or distal joints (extension of the digits). All excitatory inputs were from both large and fine myelinated fibers. A nociceptive radiant heat stimulus applied to the plantar sole was always effective in inhibiting the resting neuronal discharge. B) The second subpopulation had larger receptive fields and their background activity consisted of isolated spikes which depended on the resting position of the knee (flexion increased it while extension reduced it). Cutaneous pressure or pinch was very effective in inhibiting this activity. The inhibitory receptive fields varied in size with the largest involving both hindlimbs and ipsilateral forelimb.

The activities of VMZ cells are thus directly related to inputs from regions involved in locomotion and posture. As such a projection has not been shown in monkey (i.e. a mostly biped standing animal), it probably represents part of the system involved in the coordination of quadruped walking.

230.6 DIFFERENTIAL DIENCEPHALIC PROJECTIONS FROM THE SPINAL CORD AND THE GIGANTOCELLULAR RETICULAR AREA IN THE RAT : AN ANATOMICAL STUDY USING WGA-HRP WITH REFERENCE TO PAIN TRANSMISSION Marc PESCHANSKI*, Patrick W. MANTYH* and Jean-Marie BESSON*, INSERM U 161, 2 rue d'Alésia, Paris 75014, France and Dept of Anatomy, UCSF Medical School, San Francisco, CA 94143 USA (spon. Hugh A. Patterson)

In an attempt to define the actual sites of projection of two pathways involved in pain transmission, i.e. the spino-thalamic tract and the spino-reticulo-thalamic tract, an aqueous solution of wheat-germ agglutinin conjugated to horseradish peroxidase (WGA-HRP, 10 % Sigma) was iontophoretically injected through a glass micropipette using dc positive current either in the grey matter of the cervical or lumbar enlargement of the spinal cord, or in the nucleus reticularis gigantocellularis of the medulla (NGC) in male Sprague Dawley albino rats. 24 to 48 hours later, animals were killed with an overdose of sodium pentobarbitone, perfused transcardially with paraformaldehyde (1%) and glutaraldehyde (3%) in phosphate buffer (pH 7.4, .1M) and the brain cut frozen in 60 µm sections. Alternate sections were reacted for HRP using tetramethyl benzidine as a chromogen.

Spinal projections to the diencephalon were observed contralaterally in the lateral portion of the ventrobasal complex, the ascending branch of the intralaminar nucleus centralis lateralis and in the dorsal part of the nucleus submedius. In contrast, other than a light labelling in few ventral and medial thalamic structures, major projections of the NGC neurons were to be to the intralaminar nuclei, all of them receiving moderate density of labelling. In particular, in all cases, the area lateral to the parafascicular nucleus and defined in the present study as the nucleus center median contained numerous thin dividing fibers and dots of reaction product suggesting the presence of terminal boutons.

These anatomical data and the different electrophysiological characteristics of the thalamic neurons recorded in the same species in the ventrobasal complex (Guilbaud et al.,Pain 8,1980, 303-318) and in the posterior intralaminar region (Peschanski et al.,Exp.Neurol.,72,1981,226-238) i.e. in the sites of projection of the spino-thalamic and of the spino-reticulo-thalamic tracts respectively, are suggestive of a distinct role of the two pathways in pain transmission. The thalamic ventrobasal complex and the spino-thalamic system is possibly involved in the sensory-discriminative aspects of pain transmission and the nucleus center median and the spino-reticulo-thalamic system is possibly involved more in motor and/or behavioral responses related to pain.

230.7 NOCICEPTIVE NEURONS IN THE VENTRAL POSTERIOR THALAMUS OF THE AWAKE SQUIRREL MONKEY. T. J. Morrow and K. L. Casey. Neurology Research Laboratories, V.A. Medical Center, Ann Arbor, MI 48105.

Previous investigations of the ventral posterior (VP) thalamus in unanesthetized primates have failed to find neurons that respond differentially to noxious somatic stimuli. This study examines the responses of VP neurons to a variety of innocuous and noxious cutaneous somatic stimuli in the awake, partially restrained squirrel monkey.

Receptive fields and response properties were determined for 85 of 102 single neurons recorded from the ventral posterior lateral (VPL) and ventral posterior medial (VPM) nuclei of the thalamus in the awake squirrel monkey. These somatically responsive neurons typically discharged at rates of 1 to 20 pulses/second in the absence of intentional stimulation, and responded to innocuous or noxious, mechanical, somatic stimuli. All but 12 neurons with midline or bilateral receptive fields on the tail, responded to stimuli applied to a small fraction of a limb, the tail, trunk or face, contralateral to the recording site. Most neurons responded maximally to innocuous somatic stimuli such as hair movement (N=41) or light touch of the skin (N=38). Noxious cutaneous stimulation elicited no greater response from these neurons than innocuous stimuli.

Nine neurons, recorded from the lateral posterior part of VPL or the ventral posterior VPM, responded maximally to noxious mechanical somatic stimuli. These cells also discharged spontaneously at rates of 2 to 10 pulses/second and responded to light tactile stimulation of the skin, but not to hair movement. The most effective stimulus in each case was skin pinch sufficiently strong so to elicit withdrawal and occasionally vocalization. Noxious heat was no more effective than innocuous touch. Neurons responding exclusively to noxious stimuli were not found.

The neural activity evoked by noxious stimuli was not due to activation of central motor pathways. Active movements that were independent of the stimulus were not associated with unit discharge. Furthermore, passive limb movements, joint manipulations and muscle palpation did not evoke unit activity.

These studies have shown for the first time that, in the awake, behaving primate, there is a population of VPL/VPM neurons that is differentially responsive to noxious stimuli delivered to small, well-defined contralateral receptive fields. This finding complements and expands on the work of others in the anesthetized monkey and cat. Supported by the Veterans Administration and NIH Grant NS 12581.

230.8 CONVERGENCE OF PROJECTIONS FROM TOOTH PULP AND PERIODONTAL LIGAMENT ON SINGLE NEURONS IN FELINE NUCLEUS CENTRUM MEDIANUM. K.V. Anderson, N.F. Capra, H. Hirata and T. Jones*. Dept. of Anatomy, Univ. Mississippi Med. Ctr., Jackson, MS 39216.

Previous studies in our laboratory have shown that neurons in thalamic nucleus centrum medianum (CM) can be driven from the maxillary and mandibular teeth and from sites within the caudal zone of nucleus gigantocellularis in the brain stem reticular formation. The present studies demonstrate that CM neurons that respond to tooth pulp (TP) activation may also respond to activation of periodontal ligament (PDL) receptors.

Single cells were isolated in CM (A 6.5 to 8.0, L 2.0 to 4.0, H 2.5 to -2.5) of 10 pentobarbital anesthetized cats using tungsten microelectrodes. Cardiac rate, expired gases and body temperature were monitored throughout each experiment. CM neurons were identified by providing electrical stimulation to maxillary or mandibular canine tooth pulps and by mechanically tapping the surface of the canine teeth with a relay driven force applicator.

In the present studies, 100 neurons in CM were isolated which responded vigorously to PDL stimulation associated with the canine teeth. These cells responded with a latency that ranged from 5 to 30 msec. with an average latency of 21 msec. Ten of the cells that responded to PDL stimulation also responded to TP stimulation with latencies that ranged from 6 to 40 msec., with an average latency of 32 msec. All neuronal responses in CM to TP or PDL stimulation were excitatory and in each case the response latency following PDL stimulation was less than that following pulpal stimulation. In addition, neurons receiving converging sensory information showed more vigorous responses to pulpal stimulation than to PDL stimulation. Most CM neurons were more sensitive to ipsilateral than to contralateral stimulation, although many neurons could be activated bilaterally. In general, CM neurons sensitive to the maxillary teeth were located dorsally in the nucleus and those sensitive to mandibular teeth were located ventrally.

The present study identifies a population of midline thalamic neurons that receive convergent sensory information from both intrapulpal and extrapulpal receptors. These receptors are known to also provide inputs to various portions of the spinal trigeminal complex and to portions of the spinal cord. Taken together, these observations demonstrate that TP and PDL dental stimuli elicit convergent responses at several levels of the central nervous system and may have complex interactions within these nuclear regions. While the functional significance of this convergence in CM is, as yet, unknown, we would like to suggest a role for this midline system in sensory-motor integration and reflex functions.

230.9 REACTIONS OF CLAUSTRAL SINGLE CELLS TO THE TOOTH PULP STIMULATION IN CATS. P.J. Jastreboff*, M. Sikora*, A. Frydrychowski*, and P. Sloniewski* (SPON: R. Small). Dept. of Neurophysiology, Nencki Institute of Experimental Biology, 3 Pasteur Street, 02093 Warsaw, Poland.

Publications describing single unit recording from claustrum are scarce. Responses of claustral single cells to somatic, visual and acoustic stimulation have been recently reported. Taking into account the hypothesis of claustrum as a sensory regulator, which may prevent some types of inputs from reaching certain targets in the cortex, studies of the occurence of input of pain related information into the claustrum may provide valuable data towards this hypothesis. The tooth pulp stimulation was used as it can be easily controlled and can be taken as being nearly pure painful. A further point which needs to be clarified is whether the claustrum obtains afferents only from the cerebral cortex or from subcortical structures as well. In order to compare our results with previously published data the electrical stimulation of limbs was used as a parallel stimulation.

Experiments were carried out on 8 cats anesthetized with chloralose. The single unit activity was recorded from the central part of the claustrum. The majority of cells responded in a similar manner to tooth pulp and paws stimulation but there were cells with clear preference to a given type of stimulation.

Latencies of reactions evoked by tooth pulp stimulation were statistically significantly shorter than evoked by limb stimulation and in the former case latencies as short as 8 ms were observed. It is postulated that the central region of claustrum receives the projection from tooth pulp, and that part of it is coming not through the cerebral cortex but directly from the subcortical structures.

Research is supported by Project 10.4.1.01.3 of the Polish Academy of Sciences.

230.10 THE EFFECT OF SENSITIZATION AND NEMBUTAL ON THE RESPONSES OF PRIMATE SI CORTICAL NOCICEPTIVE NEURONS. D.R. Kenshalo, Jr. and W.C. Perkins*. Divisions of Neurbiology and Neurosurgery, Barrow Neurological Institute, St. Joseph's Hospital and Medical Center, Phoenix, AZ 85013.

Past research has shown that some neurons in areas 3b and 1 are responsive to noxious mechanical and thermal stimuli. Manipulations that alter the perception of painful stimuli should change the response characteristics of these neurons. We recorded from single neurons in SI cortex in young adult macaque monkeys (Macaca fascicularis). The animals were anesthetized with alpha-chloralose and maintained with supplements of sodium pentobarbital. Single units were isolated using tungsten microelectrodes. The cutaneous receptive fields were tested with innocuous and noxious stimuli. Innocuous stimuli included hair movement, light touch and pressure. Noxious stimuli applied to the skin included pinching and ascending noxious heat pulses (from an adapting temperature of 35°C to 43°, 45°, 47° and 50°C).

Repetition of the ascending sequence of noxious heat stimuli revealed a sensitization of the responses of 12 cortical nociceptive neurons. The threshold for a response to noxious heat was lowered and the responses to suprathreshold noxious heat stimuli were enhanced. The relative amount of sensitization of the response to noxious thermal stimulation exhibited by cortical nociceptive neurons was greater than either spinothalamic or thalamic nociceptive neurons.

The influence of Nembutal on the response of cortical nociceptive neurons was studied in 5 experiments. In two instances a bolus injection of 5 mg/kg of Nembutal completely eliminated responses to noxious thermal stimuli up to 55°C. In three other instances injections of Nembutal increased the threshold for a response from 50° to 55°.

Based on these findings, cortical nociceptive neurons respond in a consistent manner to some of the manipulations that are capable of altering the perception of painful stimulation.

Supported by NSF grant BNS-8217728.

231.1 THE ROLE OF THE A7 CATECHOLAMINE GROUP IN THE MODULATION OF PAIN PERCEPTION . J. Sagen and H.K. Proudfit. Dept. of Pharmacology, Univ. of Illinois at Chicago, Chicago, IL 60680.

Microinjection of alpha-adrenergic antagonists such as phentolamine (PTL) into the nucleus raphe magnus (NRM) produces potent analgesia. This suggests that neurons in the NRM are tonically inhibited by noradrenergic (NA) neurons and that blockade of this NA input disinhibits (activates) NRM neurons resulting in analgesia. Such analgesia appears to be mediated by bulbospinal NA neurons since it can be blocked by intrathecal injection of NA antagonists or by selective depletion of spinal cord NE produced by intrathecal 6-OHDA. These findings suggest that NRM neurons activate descending NA neurons which inhibit the transmission of nociceptive information at the level of the spinal cord. Although the location of these spinally-projecting NA neurons is not known, the A7 catecholamine group has been shown to project to the spinal cord. The purpose of the present studies was to determine whether the A7 nucleus is the origin of the bulbospinal NA pathway involved in mediating the analgesia produced by activating neurons in the NRM. To test this proposal, the effect of electrolytic lesions of the A7 nucleus on nociceptive threshold and the capacity of PTL injected into the NRM to produce analgesia was determined.

Female rats (225-250 g) were tested using the tail flick test to determine baseline nociceptive threshold. Animals then received either bilateral, unilateral, or sham electrolytic lesions of the A7 nucleus and a microinjection guide cannula aimed at the NRM was implanted under pentobarbital anesthesia. Tail flick latencies (TFL) were determined 1, 7, and 14 days following the lesion. At 14 days, PTL (10 ug in 0.5 ul of saline) was injected into the NRM and TFLs were determined 10, 20, and 30 min following the injection. Lesion and injection sites were verified histologically. Animals with either sham or unilateral A7 lesions exhibited no alterations in TFLs at 1, 7, or 14 days following the lesion. In contrast, animals with bilateral A7 lesions showed marked hyperalgesia 1 day following the lesion and partial recovery by 14 days. PTL injected into the NRM produced potent analgesia in animals with sham A7 lesions, but this analgesia was blocked in animals with either bilateral or unilateral A7 lesions. Spinal cord NE content in animals with unilateral or bilateral A7 lesions decreased to 73% and 60%, respectively, of that determined in sham-lesioned animals.

These studies support the suggestion that the spinally-projecting catecholamine neurons located in the A7 nucleus are involved in mediating the analgesia produced by activating neurons in the NRM. (This work was supported by USPHS Grant 18636 and the PMA Foundation).

231.2 HYPOALGESIA PRODUCED BY MICROINJECTION OF CARBACHOL INTO THE NUCLEUS RAPHE MAGNUS IS NOT DUE TO BLOOD PRESSURE CHANGES. M. S. Brodie and H. K. Proudfit. Dept. Pharmacology, Univ. of Illinois at Chicago, Chicago, IL 60680.

Previous reports from this laboratory have presented evidence for a functional cholinergic influence on neurons of the nucleus raphe magnus (NRM) involved in pain modulation. Microinjection of carbachol, a cholinergic agonist, into the NRM produces a profound hypoalgesia. It has been suggested that this suppression of pain perception is produced by the inhibition of incoming pain information at the spinal level. However, brainstem nuclei at the level of the NRM are thought to be involved in control of blood pressure, and elevation of blood pressure has been shown to cause a decrease in pain sensitivity. The purpose of this study was to determine whether the analgesia produced by microinjection of carbachol in the NRM is due to changes in blood pressure.

Sprague-Dawley derived rats(250-350 gm) were implanted with guide cannulae directed toward the NRM one week prior to blood pressure measurement or behavioral testing. In the first set of experiments, rats were anesthetized with urethane(1200 mg/kg, I.P.) and the carotid artery was cannulated. Body temperature was maintained at 37^{o}C, and a stable baseline blood pressure was obtained at least 15 min prior to microinjection of carbachol. Blood pressure was measured continuously for 60 min after microinjection into the NRM. Microinjection of carbachol (2.5 ug in 0.5 ul saline) was made at a rate of 0.5 ul/min using a Harvard pump. Microinjection of carbachol lateral or dorsal to the NRM produced an increase in mean arterial blood pressure (MAP) of 30.0 +/- 10.4 mmHg within 15 min. Blood pressure returned to normal within one hour. In contrast, microinjection of carbachol directly into the NRM produced no increase in blood pressure.

To further dissociate the interaction of blood pressure and pain sensitivity, the effect of hexamethonium, a ganglionic blocker, on carbachol-induced elevation in tail flick latency was tested. Pharmacological blockade of sympathetic ganglia should eliminate centrally mediated increases in blood pressure. Tail flick latency was assessed before any drug administration, 25 min after hexamethonium (2.8 mg/kg, I.P.), and at intervals after carbachol was microinjected into the NRM. Hexamethonium alone had no effect on tail flick latency, and the ganglionic blockade produced by hexamethonium did not attenuate the tail flick latency increase produced by carbachol microinjected into the NRM.

These data indicate that while brainstem cholinergic systems are involved in blood pressure modulation, the hypoalgesia produced by microinjection of carbachol into the NRM is not due to blood pressure elevation. Supported by USPHS Grant NS 18636.

231.3 EFFECTS OF NALOXONE AND ATROPINE ON SOMAN INDUCED ANTINOCICEPTION AND CENTRAL NEUROCHEMICAL CHANGES. J.A. Romano and T.-M. Shih, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010.

Irreversible cholinesterase inhibitors have been shown to produce antinociception on the rat hot plate test (HP) and to produce elevations of acetylcholine (ACh) and choline (Ch) in brain. The present experiment examined: (1) the effect of soman on HP, (2) the possible antagonism of soman effects by atropine sulfate (ATR) or naloxone hydrochloride (NAL) pretreatment, and (3) the possible neurochemical link between production of HP antinociception and changes in rat brain ACh and Ch. HP latency (in sec) to hind paw lick or foot thump was recorded with HP temperature at 56.4^{o}C and a 30 sec cutoff. Animals were tested on HP prior to injection of saline (SAL), ATR (1.1 or 2.2 mg/kg, i.p.) or NAL (3.0 or 10.0 mg/kg, i.p.). Five min following pretreatment, animals were injected with SAL or soman (69 or 80 µg/kg, i.m.) and retested on HP 30 min later. Data were reported in terms of change from baseline HP latency (sec). Immediately after the second HP test animals were killed by focused microwave irradiation. ACh and Ch were analyzed in brainstem (B), cerebral cortex (C), hippocampus (H), midbrain (M), cerebellum (R), and striatum (S) by the gas chromatograph/mass spectrometric method (Psychopharmacology 78: 170, 1982). Soman (80 µg/kg) produced significant HP antinociception which was not antagonized by either dose of ATR but was facilitated by both doses of NAL. Neither ATR nor NAL treatment alone had any effect on HP latency scores. At an analgetic dose of soman (80 µg/kg), ACh was elevated in M (174%) and Ch in B and S (239% and 169%, respectively). Elevations of ACh and Ch produced by soman in these brain areas were not affected by any dose of ATR pretreatment. However, both doses of NAL significantly enhanced soman-produced elevation of Ch in S. Neither ATR nor NAL treatment alone had any effect on ACh or Ch in any of the brain areas studied. The results of the present study indicate that: (1) soman produces antinociception on HP, (2) this antinociception is facilitated by NAL pretreatment but is not affected by doses of ATR used in the present study, and (3) soman produces elevations of rat brain ACh and Ch levels which are not affected by ATR but Ch levels are potentiated by NAL, suggesting a possible relationship between production of antinociception on the one hand and changes in neurochemical substrate on the other. These data support previous findings that brain Ch levels are influenced by the presence of opiate compounds (Fed. Proc., 41, 1303, 1982) and that brain Ch levels may play a role in the modulation of HP antinociception (Comm. Psychopharm., 1, 519, 1977).

231.4 ACETYLCHOLINESTERASE (AChE) POSITIVE CELLS AT THE ORIGIN OF THE DORSOLATERAL FUNICULUS (DLF) IN THE RAT VENTRAL MEDULLA. J.N. Johannessen*, L.R. Watkins, F. Eckenstein* & D.J. Mayer, Lab. Clinical Science, NIMH, Bethesda, MD 20205 (JNJ), Dept. Physiol. Biophys., Med. Coll. Va./Va. Commonwealth Univ., Richmond, VA 23298 (LRW, DJM), Dept. Neurochem., Max Planck, Munich, Germany.

Destruction of the spinal cord dorsolateral funiculus attenuates or abolishes analgesia produced by opiates, brain stimulation or environmental manipulations. Yet little is known about the neurochemical makeup of descending pathways within the DLF. While spinal depletion of serotonin or norepinephrine does attenuate environmentally induced analgesia, cells containing these neurotransmitters do not constitute a significant portion of the cell population which descend via the DLF. Recently, analgesia induced by intense somatic stimulation has proved susceptible to muscarinic antagonists. This result, coupled with the similar distribution of cells exhibiting AChE activity and those which descend via the DLF, suggests that DLF projecting cells within the ventral medulla may be cholinergic or cholinoceptive.

To identify cholinergic or cholinoceptive bulbospinal DLF projecting cells, discrete unilateral placements of HRP gel were made in the cervical DLF. After perfusion and sectioning, the tissue was stained for retrograde HRP and either AChE activity or choline acetyltransferase (ChAT) immunoreactivity using a rabbit antibody directed against an immune-complex consisting of pig ChAT and a monoclonal antibody.

At the level of n. raphe magnus, considerable numbers of DLF projecting cells were found to contain AChE activity. In various sections, as little as 15% or as many as 70% of the DLF projecting cells were found to be double labelled. No particular patterns of double labelled cells were found. They intermingled with non-AChE containing DLF cells both medially and laterally.

In contrast, no DLF projecting cells were found to be positive for ChAT. In contrast to the cells of the facial nucleus (n. VII) which were darkly stained for ChAT, medial portions of the ventral medulla were devoid of staining.

Taken at face value, these results suggest that a portion of ventral medullary DLF projecting cells are cholinoceptive, but not cholinergic. Absolute validation of this conclusion must be confirmed by electron microscopic observation of HRP-AChE positive cells. However, these results do provide an anatomical basis for a cholinergic link, either spinal or supraspinal, in the production of analgesia. This work supported by PHS grant DA 00576 to DJM.

231.5 ACTIVATION OF A CHOLINOCEPTIVE PONTINE AREA CONTRIBUTES TO ENVIRONMENTALLY INDUCED NOCICEPTIVE SUPPRESSION. Y. Katayama*, L.R. Watkins, D.P. Becker, and R.L. Hayes (SPON: Daniel Bossut). Depts. Neurosurgery and Physiology & Biophysics (LRW), Med. Coll. VA/Virginia Commonwealth Univ., Richmond, VA 23298.

We have recently demonstrated that muscarinic cholinergic activation of the lateral part of the cholinoceptive pontine area (dorsal and ventral parabrachial region, PBR) produces non-opiate analgesia in the cat (Neurosci. Abstr., 8:619). In the present study, we have examined the possibility that PBR functions physiologically to modulate pain sensitivity.

This study involved two separate experiments. First, we investigated the effects of pharmacological manipulations of cholinergic systems in rats on footshock induced analgesia (FSIA), a previously described model of endogenous nociceptive suppression (Science, 216:1185). Analgesia (tail flick test) induced by brief hind-paw shock was found to be markedly attenuated ($p < .0001$) by systemic scopolamine (2 mg/kg, i.p.), a muscarinic antagonist, but not by intrathecal scopolamine (0.1 mg), systemic mecamylamine (2 mg/kg, i.p.) or systemic methylscopolamine (2 mg/kg, i.p.). Since this non-opiate FSIA is not affected by decerebration at the intercollicular level (Science, 216:1185), analgesias induced by brief hind-paw shock and cholinergic activation of PBR both involve non-opiate, muscarinic cholinergic mechanisms located in the brainstem. Secondly, we examined the effects of microinjection of the muscarinic antagonist atropine (4.0 µg in 1.0 µl, bilat.) into PBR on endogenous nociceptive suppression in the cat (tail flick and calibrated pinch tests). Animals not habituated to the novel testing environment commonly showed low pain responsivity (prehabituation nociceptive suppression, Pharmac. Biochem. Behav., 10: 481). This nociceptive suppression was significantly reduced ($p < .005$) by atropine microinjection into PBR but not by atropine microinjection into non-PBR sites or microinjection of vehicle alone into PBR. Atropine microinjection failed to reduce pain responsivity in the animals repeatedly habituated to the testing environment. However, presentation of novel auditory and visual stimuli reliably replicated low pain responsivity even in these animals. Atropine microinjection into PBR again reduced this nociceptive suppression ($p < .005$). In contrast, while we found that food-deprived animals reproducibly showed marked nociceptive suppression during consummatory behaviors, this form of nociceptive suppression was not significantly attenuated by atropine microinjection into PBR.

These data indicate that the lateral part of cholinoceptive pontine inhibitory area may function physiologically to inhibit nociception in response to novel environmental events. Supported by PHS Grant DA00576 and NIH Grant NS12587.

231.6 ENCEPHALOSPINAL ANALGESIA SYSTEMS: NORADRENERGIC COMPONENT DOES NOT DESCEND IN THE DORSOLATERAL FUNICULUS (DLF). E.F.S. Kaufman, R.M. Fay, J.N. Johannessen, L.R. Watkins, & D.J. Mayer. Dept. of Physiology, Medical College of Virginia, Richmond, VA 23298 and Laboratory of Clinical Science, NIMH, Bethesda, MD 20205.

Opiate analgesia following either morphine microinjection into the periaqueductal gray or brief front paw shock of rats utilize pathways descending in the DLF. Since both of these opiate analgesias are attenuated by spinal norepinephrine (NE) depletion, the question arises whether NE fibers descend the cord in the DLF.

Brainstem neurons which project down the DLF were identified by making discrete unilateral placements of HRP gels into the cervical DLF of male rats. After two days, rats were perfused with 4% paraformaldehyde; brains were sectioned at 20um and reacted with diaminobenzidene/nickel ammonium sulfate to form cytoplasmic black granules in HRP-filled cells. A standard peroxidase anti-peroxidase reaction using goat antibody (Ab) to bovine dopamine B hydroxylase, a precursor of NE, was utilized to stain NE containing cells a translucent brown. Double-labeled (DL) cells were indicative of NE cells descending the DLF.

Almost no DL cells were found. Retrogradely labeled cells generally exhibited a different distribution from Ab stained cells. As previously reported, retrogradely labeled cells were concentrated in the paralemniscal region and the nucleus raphe alatus. In contrast, over the same rostro-caudal extent, most Ab labeled cells appeared in the locus coeruleus (LC), A5, and A7. It is important to note that A7 cells are not contained whithin the paralemniscal group as previously hypothesized. Occasionally, cells from the two groups would intermingle in an intermediate zone. However, at most, one or two cells per section were DL in a population of about 40 single labeled cells. Control studies revealed that the Ab and retrograde methods did not alter the labeling patterns of one another. In contrast to discrete DLF placements, brainstems of rats in which whole cord HRP injections were made exhibited many DL cells in areas such as the LC and A5.

These results indicate that descending pathways utilizing NE lie outside the DLF. Similarly, descending serotonergic pathways appear to lie largely outside the DLF. Since analgesia can be attenuated by destruction of these encephalospinal monoaminergic neurons or by lesions of the DLF, it is obvious that simultaneous activation of pathways within and outside of the DLF are necessary for full analgesic effects.

This research was supported by Grant # 1F32NSO7151 and Grant # DA 00576.

231.7 FUNICULAR PROJECTIONS OF THE PONTINE CATECHOLAMINERGIC FIBERS INNERVATING THE LUMBAR SPINAL CORD OF THE CAT. R. T. Stevens, C. J. Hodge, Jr., and A. V. Apkarian. Dept. of Neurosurgery, Upstate Medical Center, Syracuse, NY 13210.

Stimulation of the dorsolateral pons (DLP) in areas thought to provide the primary catecholaminergic (CA) innervation to the lumbar spinal cord is capable of producing potent inhibition of cutaneous inputs onto dorsal horn interneurons. These DLP regions include the nucleus locus coeruleus (LC), subcoeruleus (SC), the parabrachial medialis (PBM), and the Kolliker-Fuse nucleus (KF), of which the Kolliker-Fuse nucleus is the primary source of CA innervation to the spinal cord of the cat. This study was performed to determine the funicular course of the CA fibers supplying the lumbar spinal cord.

Multiple unilateral injections of the retrogradely transported fluorescent dye, Evans Blue, were made throughout the lumbar enlargement of the cat following various thoracic cord lesions. The lesions included either bilateral dorsolateral quadrant (DLQ) lesions or bilateral ventrolateral quadrant (VLQ) lesions. After a 4 day survival period, brain stem tissue was processed with glyoxylic acid for CA histofluorescence and spinal injection sites were verified histologically. With this procedure retrogradely labeled cells (EB), CA containing cells (CA) and double labeled cells (CA-EB) could be identified in the same section and their funicular course at the thoracic level determined.

When VLQ's were spared, large numbers of CA-EB cells within LC and SC appeared near control levels with bilateral projection with an ipsilateral predominance. EB cells within LC and SC were fewer than control on both sides of the brain stem. Within the Kolliker-Fuse nucleus, the number of CA-EB cells appeared slightly reduced, bilaterally, when compared with control animals. As in the control, there are very few retrogradely labeled, non-CA containing cells within KF.

When only the DLQ was spared, no double labeled, CA-EB cells and few EB cells were observed within LC. In SC and KF a significant number of double labeled cells were identified primarily ipsilaterally. Throughout the DLP, the number of single labeled, EB cells was fewer than control. Within PBM and PBL there are few EB or CA-EB cells even in control animals, therefore, their funicular projections were not determined.

The results show that in the cat, CA containing cells in LC project solely through the ventral and ventrolateral quadrants, while CA containing cells in SC and KF project primarily within the VLQ, however, each has a significant projection through the DLQ as well. Non-CA, retrogradely labeled cells within the DLP travel throughout the lateral and ventrolateral funiculi.

231.8 DORSOLATERAL FUNICULUS PROJECTIONS IN THE MONKEY (M. fasicularis) AS DEMONSTRATED BY FLUORESCENT RETROGRADE TRANSPORT. S.M.Carlton*, J.M.Chung, W.D.Willis (SPON: G.Russell). Mar.Biomed.Inst.,Physiol. & Biophys. and Anatomy, Univ. TX Med. Br., Galveston, TX 77550.

Impulses descending in the dorsal quadrant of the lateral funiculus (DLF) can inhibit noxious input at the spinal level. Lesion experiments demonstrate that analgesia produced by morphine, electrical brain stimulation, vaginal stimulation and stress are all dependent on the integrity of the DLF. The anatomy of DLF-projecting systems has been reported in both rat and cat. However, cell bodies projecting to lumbar cord via the DLF have yet to be described in the monkey. The retrogradely transported fluorescent marker granular blue (GB) was used to label cells with DLF projections in the monkey, identifying neuronal populations which could mediate antinociceptive mechanisms.

To ensure discrete placement of GB within a cord funiculus, it was incorporated into a polyacrylamide gel (5% wt/vol). A laminectomy in L2-L3 region was performed in 3 monkeys, the DLF surgically cut, and a pellet of GB gel implanted. Following 8-13 days, the animals were perfused and the brainstems and cords immediately cut (35 and 50µm, respectively) and mounted. The sections were viewed with a microscope equipped for epifluorescence. Every 5th section was drawn with an X-Y plotter and all labeled cells mapped. Boundaries and landmarks were drawn in from corresponding cresyl violet stained sections.

Following unilateral DLF implants, an abundance of retrogradely labeled cells were observed on midline in n raphe (r) magnus and n r obscurus, mainly ipsilaterally in n medullae oblongatae centralis (CN) subventralis, n paragigantocellularis lateralis, mainly contralaterally in red n, and bilaterally in n reticularis (R) gigantocellularis. A fair number of labeled cells was observed mainly ipsilaterally in the lateral reticular n and Edinger-Westphal n; contralaterally in paralemniscal RF; bilaterally in locus coeruleus (LC), sub LC, caudal and medial pontine RF, paramedian medullary RF, dorsolateral to the facial n and in n r pallidus. Scattered labeling was present in the ipsilateral gracile and cuneate n, and Kolliker-Fuse n; contralaterally in lateral, medial and inferior vestibular n; bilaterally in CN subdorsalis, n solitary tract, in the area around the inferior olive, dorsal to the superior olive, n cuneiformis, midbrain tegmentum and n Darkschewitsch.

This information establishes possible anatomical substrates underlying descending nociceptive mechanisms in the primate. All of these nuclei have been previously described as having DLF projections in cat and rat, thus it can be inferred that manipulation of the DLF in monkey will result in sequelae similar to those seen in these lower species. (Supported by NIH grants NS 07062, NS 18830, NS 09743 and NS 11255 and a grant from The Moody Foundation.)

231.9 THE EFFECTS OF PENTOBARBITAL ON NALOXONE REVERSIBILITY OF RAPHE INHIBITION OF SPINAL DORSAL HORN NEURONS. Y.Hori*, K.Endo*, J.L. Steinman*, and W.D.Willis. Marine Biomed. Inst. & Depts. of Physiol. & Biophys. & Anatomy, Univ. TX Med. Branch, Galveston, TX 77550.

An interaction between the opiate antagonist naloxone and barbiturates has been reported by several authors (Doi et al., Neurosci. Ltrs. 32:81, 1982, etc.). The purpose of the present study was to investigate the effects of sodium pentobarbital on naloxone reversibility of raphe inhibition of spinal dorsal horn neurons.

In 15 unanesthetized cats decerebrated at a supracollicular level, the activity of single neurons backfired from an electrode placed at the C3 level was recorded from the lumbosacral spinal cord dorsal horn using glass microelectrodes containing a carbon microfilament. A monopolar stainless steel electrode was inserted into the medulla at 2.5-4.5 mm rostral from the obex and 4-7 mm deep to the surface of the IVth ventricle. Stimulation sites were marked by lesions that were checked histologically after the experiments. Stimulation in the NRM was by 500 ms trains of monophasic square wave pulses (duration: 0.1-0.5 ms; current strength: 25-200 μA; 333 Hz).

We observed that the responses to noxious stimuli (pinch with forceps applied to the receptive field) were inhibited by NRM stimulation in 13 neurons. In 12 cases, the inhibition was antagonized by naloxone (0.05 mg/kg, i.v.). The effects of naloxone on the NRM inhibition of the response to innocuous stimuli (brushing applied to the receptive field) were tested in 5 neurons; the inhibition was naloxone reversible in 2 cases.

In 4 cats, after recovery from the effects of naloxone (60-90 min. after injection of naloxone), sodium pentobarbital was administered intravenously in doses up to 10 mg/kg. The pentobarbital was infused slowly to prevent hypotension. After injection of pentobarbital, the effects of naloxone on NRM inhibition were retested. In 11 trials using different strengths of NRM stimulation on 4 neurons, naloxone reduced the percent inhibition produced by NRM stimulation from 65% to 47% before pentobarbital, but only from 62% to 61% after pentobarbital. The effects of naloxone were significantly smaller in the presence of pentobarbital as compared to before injection of pentobarbital ($p < 0.05$).

These findings seem to indicate that pentobarbital interferes with the action of naloxone in reversing NRM inhibition of nociceptive responses of dorsal horn neurons, although the mechanism for this effect is not clear.

(Supported by NIH grants NS 09743 and NS 11255 and a grant from The Moody Foundation.)

231.10 CHARACTERIZATION OF INHIBITION OF THE TAIL FLICK REFLEX BY STIMULATION IN THE MIDBRAIN AND MEDULLA IN THE PENTOBARBITAL-ANESTHETIZED RAT. J. Sandkühler* and G.F. Gebhart. (SPON: W. Kaelber). Dept. Pharmacology, Univ. Iowa, Iowa City, IA 52242.

Focal electrical stimulation (STIM) in the midbrain periaqueductal gray (PAG) and medullary nucleus raphe magnus (NRM) have been demonstrated to produce an antinociception in a variety of animals and analgesiometric tests. Only recently, however, have studies been performed examining the distribution of effective STIM sites medially and laterally in the midbrain and medulla. In this study, we examined and mapped STIM sites mediolaterally and dorsoventrally in both the midbrain and medulla in the anesthetized rat, employing inhibition of the spinal nociceptive tail flick (TF) reflex to evaluate the efficacy of STIM.

Rats were initially anesthetized with 50 mg/kg of pentobarbital ip for craniotomy and venous cannulation and subsequently maintained lightly anesthetized (corneal and flexion reflexes present) by iv infusion (.5-1.0 mg/kg/hr). TF latencies were significantly faster in the lightly anesthetized state (1.63 ± .01 vs 2.36 ± .04 sec awake), corresponding to tail skin temperatures of 39.2°C and 43.9°C respectively. Monopolar STIM was continuous 100 Hz constant current cathodal pulses of 100 μsec duration, started 10 sec before heat was applied to the tail. At some sites in the midbrain, STIM started 0, 2.5 or 5 sec before heating the tail had higher thresholds for inhibiting the TF reflex than did STIM started 10 sec before; at other midbrain sites and at medullary sites, STIM started 20 sec before heating the tail had higher thresholds. Strength-duration curves for thresholds of TF inhibition in the midbrain and medulla yielded chronaxies of 95 and 93.75 μsec, respectively, indicating that fibers of passage were likely affected by STIM. Electrode tracking through the midbrain revealed that the lowest thresholds for inhibition of the TF reflex were located in the reticular formation lateral to the PAG, extending ventrally and eventually to the midline at a point approx. 2 mm ventral to the ventral boundary of the PAG. Thresholds in the PAG or at its ventrolateral edge were higher. Thresholds for TF inhibition did not differ in the awake and anesthetized state, although some STIM sites effective in the anesthetized state were aversive at the same STIM intensities in the awake state. There was no clear differential distribution of such STIM sites in the PAG. In the medulla, the lowest thresholds for inhibition of the TF reflex were contained in a narrow band extending mediolaterally across the dorsal part of the NRM and through the nucleus reticularis gigantocellularis; thresholds were higher in the ventral 2/3 of the NRM and the nucleus reticularis paragigantocellularis. Supported by DA 02879.

231.11 LIDOCAINE BLOCKADE OF NUCLEUS RAPHE MAGNUS AND THE LATERAL MEDULLARY RETICULAR FORMATION INDICATES THE DESCENDING PATHWAYS FOR INHIBITION OF A SPINAL NOCICEPTIVE REFLEX FROM THE PAG ARE DIFFUSELY ORGANIZED IN THE RAT. G.F. Gebhart and J. Sandkühler*. Dept. Pharmacology, Univ. Iowa, Iowa City, IA 52242.

Last year at these meetings we reported that the descending pathways of inhibition of noxious-evoked dorsal horn neuronal activity in the cat produced by electrical stimulation in the periaqueductal gray (PAGS) were diffusely organized in the medulla. Lidocaine (LIDO) injected in either the nucleus raphe magnus (NRM) or the adjacent lateral medullary reticular formation (MRF) failed to affect the descending inhibition of spinal nociceptors by PAGS; LIDO blockade of both the NRM and MRF simultaneously was necessary to block the spinal inhibitory effect of PAGS (Neurosci. Abs. 8: 768). Employing an anesthetized rat preparation and inhibition of the spinal nociceptive tail flick (TF) reflex in these experiments, we first established the efficacy of PAGS to inhibit the TF reflex before systematically examining the effects of LIDO administered in the medulla on the efficacy of PAGS.

Rats were anesthetized with 50 mg/kg of pentobarbital ip and subsequently maintained lightly anesthetized by iv infusion (see Sandkühler and Gebhart, this volume). Monopolar brain stimulation (STIM) consisted of continuous 100 Hz constant current cathodal pulses (100 μsec) started 10 sec before the TF test.

The extent of the functional blockade produced by LIDO (4%, 0.5 μl) injected in the medulla revealed that the STIM threshold for inhibition of the TF reflex at the injection site in the medulla was elevated by 156% above control up to 60 min after LIDO microinjection. The STIM threshold for TF inhibition 0.5 mm distant from the site of LIDO injection was elevated 63% at the same time while the inhibitory STIM threshold had returned to control values by 30 min 1 mm distant from the LIDO injection site.

When the NRM alone or when the MRF bilaterally was blocked by LIDO, STIM thresholds for TF inhibition by PAGS were not significantly elevated (15.0 and 14.5%, respectively). When the NRM and MRF ipsilateral to the PAG electrode were blocked simultaneously by LIDO, the PAGS threshold for TF inhibition was increased 56.6%. When the NRM and MRF bilaterally were simultaneously blocked by LIDO, the PAGS threshold for inhibition of the TF reflex was increased 102.6%.

Thus, the descending pathways for inhibition of the spinal nociceptive TF reflex activated by STIM in the PAG are diffusely organized in the medulla, involving both the NRM and MRF. In the rat, unlike the cat, the contralateral MRF also appears to be involved. Supported by DA 02879.

231.12 **ROLE OF NEUROTENSIN IN THE INTERACTION BETWEEN THE PERIAQUEDUCTAL GRAY AND NUCLEUS RAPHE MAGNUS.** D. Haggard*, M.M. Behbehani, and F. Zemlan. (Spon: G. Khodadad) Dept. of Physiology, Univ. of Cincinnati Coll. of Med., Cincinnati, OH 45267

The periaqueductal gray (PAG) and the nucleus raphe magnus (NRM) are two essential components of a descending pain inhibitory system. Although the pharmacology of this system has been extensively studied, the neurotransmitter(s) that mediates the interaction between these sites remains unknown. Recent anatomical studies have shown that a significant number of neurotensin (NT) containing neurons that are located in the PAG have direct projection to the NRM. In this study we examined the possibility that NT may act as a neurotransmitter between these sites.

Male Sprague Dawley rats were anesthetized with 1-2g/kg urethane and were prepared for single unit recording from NRM (AP -12 to -12.5; L 0.0 to 0.1; D 8.9 with an electrode inserted at an angle of 20 degrees with respect to the vertical plane) using a double barrel electrode, and electrical stimulation of PAG (AP -5.8; D 5.5; L 0.8). NT was applied by pressure injection of 5 to 30 PSI. Activity of 61 neurons were recorded. As shown below, a significant number of neurons that responded to PAG stimulation did not respond to NT. Only 11 percent of NRM neurons were excited and 24 percent were inhibited by NT.

		RESPONSE TO PAG STIMULATION			
		EXC	INH	NR	TOTAL
RESPONSE TO NEUROTENSIN	EXC	5	0	2	7
	INH	7	4	4	15
	NR	24	2	13	39
	TOTAL	36	6	19	61

The result of this study indicates that NT has little effect on the activity of NRM neuron and is not the neurotransmitter between the PAG and NRM. It is possible that the neurotensinergic projection from PAG to NRM is involved in presynaptic modulation of afferents other than from the PAG to the NRM.

231.13 EFFECT OF MORPHINE MICROINJECTION INTO NUCLEUS RETICULARIS PARAGIGANTOCELLULARIS ON ACTIVITY OF NEURONS IN NUCLEUS RAPHE MAGNUS: COMPARISON OF SPONTANEOUS AND NOXIOUS-EVOKED ACTIVITY. Mary M. Heinricher and J. Peter Rosenfeld. Cresap Neuroscience Lab., Northwestern University, Evanston, IL 60201

The nucleus reticularis paragigantocellularis (PGC) of the ventral medulla has recently been implicated in the mechanisms of opiate analgesia. Electrical stimulation of, or opiate microinjection into, this region has antinociceptive effects in behaving animals. There is some evidence which suggests that this antinociceptive effect is mediated by nucleus raphe magnus (NRM) (Azami, et al., Pain, 1982). Recent studies in our laboratory have been designed to investigate the effect of PGC morphine microinjections on unit activity in NRM.

Rats were anesthetized with urethane (1.2 g/kg) and a 25 gauge guide cannula implanted above PGC. NRM unit activity was recorded using a glass micropipette. Cells responsive to noxious peripheral stimulation (noxious pinch with a toothed forceps and noxious heat delivered via a 10 ohm resistor applied to the skin) were chosen for study. Only one cell was recorded from in each animal. After the spontaneous firing rate and responses to noxious stimulation were determined, morphine sulfate (1 ug in 0.25 uL) was infused into PGC through a 31 gauge injector cannula over the course of 4 minutes. This dose was chosen because it reliably produces profound analgesia in awake animals. Following the microinjection, spontaneous firing rate and the responses to nociceptive stimulation were monitored for 30 to 60 minutes. When appropriate, morphine effects were challenged using naloxone (1 mg/kg, i.p.).

As we have recently reported (Heinricher and Rosenfeld, Brain Research, 1983), the predominant effect of PGC morphine microinjections on NRM spontaneous activity was suppression, and this effect was reversed by administration of naloxone. In contrast, preliminary findings indicate that the noxious-evoked excitation of these neurons is much less affected by PGC morphine microinjections. As a consequence, the response to noxious input relative to ongoing NRM activity is enhanced. These results suggest that the analgesic effect of PGC morphine microinjections may be mediated not by a tonic increase in descending inhibition from NRM, but by a relative increase in the nociceptive responsiveness of this system.

Supported by NIH grant GM 23696 to JPR.

231.14 INHIBITION OF SPINAL DORSAL HORN NEURONS BY STIMULATION IN BASAL GANGLIA AND AMYGDALA. S. Pretel, M.J. Guinan*, S.N. Suberg & E. Carstens. Dept. Animal Physiol., Univ. Calif., Davis, CA 95616.

Electrical stimulation at ventromedial diencephalic and forebrain sites inhibited the responses of spinal dorsal horn neurons to noxious skin heating (Fraunhoffer et al., Soc. Neurosci. Abs. 8:767, 1982). We have continued this study to determine if spinal inhibition is produced by stimulation at more lateral sites in the basal ganglia (caudate n.= Cd; globus pallidus= GP; putamen= PU) and subjacent amygdala and prepiriform area.

Responses of single lumbar dorsal horn units to noxious heat stimuli (50^{o}C, 10 s) applied to glabrous footpad skin were recorded in 24 cats anesthetized with sodium pentobarbital and ventilated with 70% N_2O. Using a medio-lateral array of bipolar stimulating electrodes, effects of stimulation (100 msec pulse trains at 100 Hz, 3/s, 25-300 μA) at a variety of lateral diencephalic or forebrain sites were expressed as the magnitude of the unit response to heat during stimulation at each site as a % of the unit control heat-evoked response in the absence of brain stimulation.

Dorsal horn unit responses to heat were reproducibly and powerfully suppressed (to 50% of control or more) by stimulation in Cd, internal capsule, amygdala and prepiriform areas bilaterally. Inhibitory sites were distributed throughout the dorso-ventral and medio-lateral extent of the amygdala and prepiriform area at anterior levels +11 to +16, with no evidence of topographic organization. Inhibition was also generated at sites in PU and GP bilaterally, but this inhibition was weaker (51-80% of control) in some animals. Strong inhibition was frequently produced at sites along tracks passing through cortex lateral to the internal capsule.

Effects of stimulation at one effective amygdalar or prepiriform site on dorsal horn unit responses to graded noxious heat stimuli were tested in 20 cases. Unit responses increased in a linear manner with graded heat stimuli. The slope of the stimulus-response function was generally reduced, usually with minor changes in response threshold (but with threshold increases of up to 5^{o}C in a few cases) during stimulation at ipsilateral (N=9 units) or contralateral (N=11) sites. Similar effects were produced by contralateral Cd stimulation in 5 units to date.

The results indicate that stimulation in basal ganglia structures classically associated with the "extrapyramidal" motor system, and in amygdala and prepiriform areas associated with the limbic system, can strongly inhibit the spinal transmission of nociceptive information. We are investigating the possible role of medial brain stem structures in mediating inhibition from these areas.

Supported by USPHS Grant NS19330-01.

231.15 FOREBRAIN ACTIVATION OF RAPHE-SPINAL NEURONS. M.J. Guinan*, S. Pretel, S.N. Suberg & E. Carstens. Dept. Animal Physiology, Univ. Calif., Davis, CA 95616. (SPON: M. McNamee).

Electrical stimulation at a variety of forebrain sites inhibited the responses of spinal dorsal horn neurons to noxious skin heating (Fraunhoffer et al., Soc. Neurosci. Abs. 8:767, 1982). Inhibition from some of these areas could be mediated via activation of an inhibitory raphe-spinal pathway. We therefore investigated the effects of stimulation at sites in the diencephalon and posterior telencephalon on single raphe-spinal neurons.

Antidromic responses of single units in the medullary nucleus raphe magnus to unilateral stimulation of the dorsolateral funiculus at an upper lumbar level were recorded in cats anesthetized with sodium pentobarbital and 70% N_2O. To date, 46 units gave constant-latency responses (range: 2.5-17.5 msec to yield conduction velocities of 15 to >100 m/sec) at high frequencies (>100 Hz) of cord stimulation, and 29 showed collision of the antidromic spike with orthodromic spikes elicited by forebrain stimulation. Most units gave orthodromic responses at latencies of 3-70 msec to single 0.1 msec forebrain stimuli, but some required repetitive stimuli (100 msec trains at 100 Hz, 3/sec, 25-500 μA) to be driven. Activity in 4 of the latter outlasted the period of brain stimulation by several seconds.

Maps of sites at which stimulation activated raphe-spinal units were constructed by systematically varying the medio-lateral and dorso-ventral position of the bipolar stimulating electrode at one anterior level (range: +9 to +15) in 12 cases. The current threshold for activating the unit, and/or the number of orthodromic spikes elicited by stimulation, were analyzed to compare the relative effectiveness of stimulation at each brain site. Units were typically activated from sites throughout the forebrain, with variations in the effectiveness of stimulation at different sites. The relative effectiveness of stimulation in a given structure varied from one unit to the next. Despite these inter-unit differences, the following sites were particularly effective in activating raphe-spinal units in nearly all experiments: amygdala, internal capsule and hypothalamic periventricular gray bilaterally. Less effective sites included the basal ganglia (caudate, putamen and globus pallidus), medial and lateral thalamic nuclei, and region of the optic tract.

The results indicate that stimulation at many sites in the forebrain known to inhibit spinal dorsal horn neurons can also activate raphe-spinal units. If the recorded raphe-spinal units are involved in spinal inhibitory mechanisms, they could mediate descending inhibition from more anterior areas.

Supported by USPHS Grant NS19330-01.

231.16 RAPHE MAGNUS LESIONS BLOCK THE RESTRAINT POTENTIATION OF MORPHINE ANALGESIA. S. J. Kelly and K. B. J. Franklin. Department of Psychology, McGill University, Montreal, Quebec, H3A 1B1.

Rats which are restrained throughout tail flick testing have a different time course of morphine analgesia than rats which are left in their home cages between unrestrained tail flick tests. Restrained rats show a peak analgesic response 30 min after a subcutaneous (s.c.) injection of morphine. Unrestrained rats show a maximum analgesic response 60 minutes after a s.c. injection of morphine and the degree of morphine analgesia never reaches that of the peak analgesic response of restrained rats. The potentiation of morphine analgesia in restrained rats was shown to be dependent upon raised serotonin synthesis via increased uptake of brain tryptophan that results from restraint stress. Since the descending serotonergic projections of the nucleus raphe magnus (NRM) are known to be involved in morphine analgesia, the possibility that the NRM mediates the restraint potentiation of morphine analgesia was tested.

A group of male Long-Evans hooded rats received electrolytic lesions (1 mA, 10 sec) of the NRM. A second group received sham operations. Fourteen days after surgery, all rats were exposed to the laboratory for 90 min every day for 6 days. On the seventh day, the rats were brought into the laboratory and half of the lesioned and sham-operated rats were restrained in wire mesh restraining tubes. The rest of the rats were left in their home cage and manually held during each tail flick test. The tail flick test consisted of recording the latency with which the rat removed its tail from 55^{o}C water. All rats received one tail flick test, were then injected with morphine hydrochloride (5 mg/kg, s.c.), and tested every 15 min for 90 min after the injection.

Lesions of the NRM had very little effect on the time course of morphine analgesia in the unrestrained rats. Both lesioned and sham-operated unrestrained rats showed maximum analgesia 60 min after the morphine injection and the degree of analgesia never reached that of the 30 min peak analgesic response in the restrained sham-operated rats. NRM lesions in restrained rats greatly reduced the 30 min peak analgesic response and made the time course of analgesia in the restrained rats comparable to that of the unrestrained rats. These results suggest the NRM mediates the restraint potentiation of morphine analgesia but is not involved in morphine analgesia itself. (Supported by NSERC Grant No. A6303.)

231.17 RESTORATION OF FUNCTION OF THE DESCENDING FIBERS FROM THE NUCLEUS RAPHE MAGNUS BY 5-HYDROXYTRYPTOPHAN IN RATS ADDICTED TO MORPHINE. R. Emmers. Dept. of Physiol., Coll. of P & S, Columbia U., New York, N. Y. 10032.

Recent experiments (Physiologist 24:25) have indicated that during narcotic withdrawal the nociceptive system undergoes sensitization. Since the fibers descending from the nucleus raphe magnus (NRM) can block excitation of the spinothalamic tract (STT) by A-delta and C-fibers (J. Neurophysiol. 45:121), and thus modify pain, it appeared likely that narcotics alter the function of the NRM fibers. This possibility was investigated with 18 rats using electrophysiological methods. Twelve of them were addicted to morphine (80-120 mg/kg/day); 6 served as morphine-naive controls. Each rat received an i.p. injection of chloralose and urethane and was prepared for a stereotactic approach to neurons of the second somesthetic region (SII) of the thalamus. There a nociceptive neuron was identified by its unique spacing of spike potentials emitted in response to pricking the foot with a pin. Single-pulse stimulation of the sciatic nerve evoked the same response as pinpricks, but innocuous stimuli reorganized the spacing of the late spike potentials. Only neurons exhibiting this differential response were regarded as nociceptive; their response activity was accumulated on a digital computer to obtain post-stimulus time histograms. Similar to responses evoked in single STT neurons (J. Neurophysiol. 45:121), histograms of the thalamic neuron activity had peaks revealing the STT relay of the A-alpha, A-delta, and C-fiber input (Emmers, R. Pain: A Spike-Interval Coded Message in the Brain. Raven Press, 1981). With the control animals, the A-delta and C-fiber activity peaks were promptly suppressed by stimulation of the NRM (a 100-msec pulse train at 300/sec, 0.5 msec pulse width, terminated 20 msec before a single-pulse stimulation of the sciatic nerve, repeated at 2 sec intervals). With the addicted rats the A-delta and C-fiber activity peaks were dramatically elevated during naloxone precipitated narcotic withdrawal, and NRM stimulation diminished them only slightly. However, 5-HTP (3.5 mg/kg i.v.) promptly returned the augmented activity peaks to the control level and NRM-stimulation obliterated them. These results suggest that during morphine addiction the serotonin supply in the NRM fibers is gradually depleted, and, when naloxone lowers the spontaneous activity of the NRM pathway (unpublished observations), the blocking of the A-delta and C-fibers is lifted, resulting in sensitization of nociception. The serotonin precursor, 5-HTP, restores the synaptic function of the NRM descending fibers, reinstating the A-delta and C-fiber block. (Aided by grant DA-03292 from NIDA).

231.18 MICROINJECTION OF 6-OHDA IN THE REGION OF THE NUCLEUS TRACTUS SOLITARIUS DOES NOT ALTER PAIN THRESHOLD OR ANALGESIC EFFECTIVENESS OF SYSTEMIC MORPHINE INJECTIONS IN RATS.** N. Oley, J.D. Bronzino, and C. Siok*. Psychology and Engineering Departments, Trinity College, Hartford, CT 06106.

The nucleus tractus solitarius (NTS) is known to contain opiate receptors with pain-modulating functions (Oley et al., Brain Rsch., 236:511, 1982). This region also contains catecholaminergic (CA) neurons (Chiba & Kato, Brain Rsch, 1978) whose role in pain modulation is unknown. It has been suggested that central CA pathways are directly involved in pain modulation and may also mediate the analgesic effects of systemic opiates. The present study sought to determine whether the CA neurons in the region of the NTS play a role in pain modulation and/or opiate-induced analgesia.

Sprague-Dawley rats used as their own controls were first trained to perform a fixed-ratio liminal escape (LE) task (Bodnar et al., Neurosci. Biobeh. Rev., 1974). This complex operant task assesses both the sensory/discriminative and motivational aspects of the response to nociceptive stimuli. Once a stable operant baseline performance was established, subjects were given 17 daily LE tests. On day 1 a single 10 mg/kg, i.p., morphine sulphate injection was given 30" prior to LE testing. On day 8, after LE testing, subjects received 8 ug of the CA neurotoxin 6-OHDA in .15 ul of buffered Ringers + 0.1% ascorbic acid via a 33 ga cannula aimed at the NTS. Finally, on day 17, a second 10 mg/kg, i.p., injection of morphine was administered 30" prior to LE testing.

Destruction of CA neurons in the vicinity of the NTS did not alter sensitivity to footshock or the analgesic effectiveness of systemic morphine as measured by LE task. There was no evidence of morphine tolerance in control subjects that received only morphine, and 6- OHDA had no significant effects on the nociceptive response when injected outside the region of the NTS.

Thus, our preliminary results suggest that CA neurons in the NTS do not mediate the response to painful stimuli, and do not appear to interact significantly with the opiate-mediated pain-suppressive system in NTS or elsewhere.

*Supported by NIH Grant GM27226-03

231.19 DORSAL RAPHE STIMULATION, MORPHINE AND 5-HT IONTOPHORESIS EFFECTS ON MEDIAL THALAMIC UNITS FOLLOWING NOXIOUS AND NONNOXIOUS STIMULATION C. Reyes-Vázquez, B. Prieto-Gómez* and N. Dafny. Dept. Fisiol. Fac. Med. UNAM. México. and Neurobiol. Dept. UT Med. Sch. Houston Tx. 77025.

It has been shown that responsiveness to noxious stimuli can be reduced by focal electrical stimulation of selective brain loci. One explanation of this phenomenon is the existence of descending pain supressing mechanisms at the spinal cord level. Moreover, there is evidence that pain modulation also occurs at higher CNS levels, such as medial thalamus (MT). This work examines the possibility of ascending pain supression mechanisms by analyzing the effects of dorsal raphe stimulation (DRS) and microiontophoresed morphine (MOR) and 5-HT upon the activation of units in the MT of the rat, following noxious (NS) and nonnoxious stimulation (NNS). In urethane anesthetized rats a craniectomy was performed above the MT region and bipolar stimulating electrodes implanted within the DR. An array of 5 micropipettes was used for microiontophoresis containing: (1)MOR 0.045 M pH=4.5; (2)5-HT 0.01 M pH=5.0; (3)NaCl 2 M (used as balance barrel); (4)pontamine sky blue (used to mark the electrode location) and (5)recording electrode filled with NaCl 4 M. An aligator clip placed on the rat's tail or the immersion of the tail in a water bath (50°C) were used for NS. Stroking of the tail or extremities, light taps, brushing of the hair or puffs of air, were used as the NNS. The DR was stimulated from 0.1 to 0.6 mAmp with 10-15 HZ. After 5 min. of the spontaneous activity recording the effects of NS and NNS on each unit were recorded. After this, the effects of MOR and 5-HT microiontophoresis for 60 sec. with currents of 50 nA were recorded. Finally, simultaneously drug application with NS or NNS was tested. From 137 units exhibiting spontaneous activity only 79 were affected by NS, NNS or any of the drugs tested. 18/79 cells were affected only by MOR or 5-HT. In the 61/79 MT units it was possible to identify 3 different types of units accordingly to the responses following NS or NNS. Units excited exclusively by NS=11/61; units accelerated exclusively by NNS=15/61 and mixed units that responded both to NS and NNS=35/61. DRS elicited a decrease fire in 72% of 61 units analyzed. Similar effects could also be obtained by 5-HT and MOR microiontophoresis. MOR and 5-HT induced decreases mainly in MT units activated by NS, and had lesser effects in those units which were excited exclusively by NNS. DRS and 5-HT microiontophoresis decreased the acceleration induced by NS in 63% of noxious and mixed units, and only in 12% of the units affected exclusively by NNS. MOR was less effective in blocking these responses. This data suggest that DR and 5-HT are involved in the modulation of nociceptive responses of MT, and there are some similarities between the opiod effect and the serotonergic effects on the NS responses at the level of the MT.

232.1 SPINAL LEVEL ANALGETIC EFFECTS OF SEROTONIN AND SERTALINE IN A SUBSTANCE P-BASED PAIN MODEL, P.G. Cosgrove* and C.J. Pazoles, Central Research Division, Pfizer Inc., Groton, CT 06340.

Neuronal pathways which descend from the midbrain and medulla to the dorsal horn of the spinal cord appear to modulate the response to incoming nociceptive stimuli by direct inhibition of thalamic projection neurons and by activating enkephalinergic interneurons. Serotonin is present in some of these descending fibers and may play a role in their analgetic functions. When serotonin is injected into the lumbar subarachnoid space of several species, the animals exhibit a dose-dependent analgesia to noxious thermal stimuli (Yaksh and Wilson, J. Pharmac. Exp. Ther. 208:446, 1979). Furthermore, serotonin modulating drugs have been shown to influence analgesia. Indeed, amitriptyline and other antidepressant drugs that block monoamine reuptake have been used clinically in the treatment of certain painful conditions (eg. Watson, et al., Neurology (NY)31(part 2):68, 1981).

We examined the effects of serotonin and sertraline on substance P (SP) induced bite/scratch behavior in mice. SP (20 pmoles) injected intraspinally (i.s.) into the lumbar region causes a presumably nociceptive reaction characterized by vigorous biting and scratching at the abdomen. Coadministration of serotonin (0.2 pmoles, i.s.) inhibited the bite/scratch response by approximately 50%. The serotonin antagonist methysergide (2 ug, i.s.) blocked serotonin's analgetic effect. The effect was also reversed by peripherally administered naloxone (2 mg/kg), thus implicating the endogenous opiate system in the spinal level analgesia produced by serotonin. Sertraline (CP-51,974) is a highly selective serotonin reuptake blocker, 20 times more potent versus serotonin than norepinephrine reuptake. When subcutaneously administered one hour prior to SP administration, sertraline (10 mg/kg) significantly reduced the bite/scratch response (approximately 50%). As with serotonin, methysergide (2 ug, i.s.) reduced the analgetic effect of s.c. sertraline implicating serotonin reuptake blockade in the spinal cord as the mechanism of sertraline's action. When coadministered i.s. with SP, sertraline (1.9 nmoles) had no effect on the bite/scratch response, presumably because of the time necessary for serotonin accumulation following reuptake blockade.

These results demonstrate that serotonin is capable of modulating the pain-producing effects of SP at the spinal level, perhaps by activation of an endogenous opiate system. The ability of peripherally administered sertraline to produce spinal level analgesia suggests that this drug may be useful as a centrally acting analgetic.

232.2 LOCUS COERULEUS (LC) LESIONS INCREASE THE ANTINOCICEPTIVE POTENCY OF CLONIDINE GIVEN INTRATHECALLY BUT NOT MORPHINE GIVEN IN THE PAG. M.H. Ossipov and G.F. Gebhart. Dept. Pharmacology, Univ. Iowa, Iowa City, IA 52242.

Existing evidence indicates that the LC is a source of noradrenergic projections to the spinal cord and may be involved in descending inhibition and antinociception which appear to be mediated in part by spinal norepinephrine (NE). Clonidine exerts an antinociceptive effect directly at a spinal level by interacting with α_2-adrenergic receptor sites, and also modulates LC neuronal activity. To examine the role of the LC in antinociception, bilateral electrolytic LC lesions were made in male Sprague-Dawley rats. Tail-flick (TF) and hot plate (HP; 51 and 55°C) latencies were determined at days 0, 7, 13 and 14. No significant differences in response latencies between sham and lesion groups were observed in either test on any of the days tested. The antinociceptive efficacy of morphine administered in the periaqueductal gray (PAG) in doses of 2.5 and 5.0 µg (base) on days 7 and 14 was not different when compared between sham and lesion groups in either the TF or HP tests.

On day 13, clonidine was administered intrathecally in lightly anesthetized lesion and sham animals through an implanted cannula terminating at the lumbar enlargment of the spinal cord. In lesion rats, the dose required to elicit one half the maximal increase in TF latency (i.e., ED_{50} at 4 sec) was 0.32 µg (base), while 1.67 µg of clonidine was required to elicit a similar response in sham rats. Scatchard analyses of ^{3}H-clonidine binding in the lumbar cord obtained from rats sacrificed on day 15 revealed similar binding affinities between sham (K_d = 1.93 nM) and lesion (K_d = 2.72 nM) groups. B_{max} was 31.0 fmole/mg protein in sham and 44.3 fmole/mg protein in lesion rats, indicating increased α_2-adrenergic receptor numbers in the latter group. NE content of the lumbar spinal cord was greater in sham (0.765 ± .089 µg/gm tissue) than in lesion (0.631 ± .078 µg/gm tissue) rats. Serotonin and 5-hydroxyindoleacetic acid levels in lumbar cord were not different in sham and lesion animals.

The results indicate that spinal LC projections are not essential for expression of inhibition of a nociceptive spinal reflex by morphine administered in the PAG. However, spinal projections of the LC appear to be partly involved in α_2-adrenergic receptor mediated antinociception since lesions of the LC resulted in postsynaptic α_2-receptor supersensitivity as evidenced by increases in the antinociceptive potency of clonidine and B_{max}. Supported by DA 02879 and MH 15172.

232.3 REDUCTION OF AUTOANALGESIA FOLLOWING INJECTION OF 6-HYDROXYDOPAMINE INTO THE LOCUS COERULEUS OR CISTERNA MAGNA. William T. Chance and John C. Peters* (SPON: J.A. Rosecrans). Department of Surgery, Univ. Cincinnati Med. Ctr., Cincinnati, Ohio 45267.

Analgesia elicited by acute stress and classical conditioning procedures results from behavioral activation of descending antinociceptive mechanisms. Since dysfunction of these endogenous analgesia systems may result in chronic pain problems, specification of the neurochemical mediators of the analgesia is an important goal. Our previous research demonstrating the efficacy of yohimbine in antagonizing autoanalgesia and cross-tolerance of autoanalgesia to clonidine suggests a role of norepinephrine (NE) in mediating endogenous antinociception. To permit direct investigation of this hypothesis, acquisition of autoanalgesia was assessed in rats that had been treated with the catecholamine neurotoxin, 6-hydroxydopamine (6-OHDA). In one group of adult, male, Sprague-Dawley rats (n = 7) 6-OHDA HBr (30 ug/3 ul, free base) was injected bilaterally into the locus coeruleus (LC) through a 30 ga needle employing stereotaxic techniques. In another group of rats (n = 8) 6-OHDA HBr (200 ug/20 ul, free base) was injected into the cisterna magna (CM) through a 27 ga needle. Control rats (n = 14) were treated with all experimental procedures, except no injections were made into the LC. One week later basal tail-flick latencies were determined for all rats. The intensity of the heat source was adjusted to elicit basal response latencies of approximately 3 sec and an 8 sec cut-off criterion minimized tail-damage. All of the 6-OHDA-treated and half of the control rats were subjected to footshock (1 ma, 15 sec). Tail-flick latencies were determined again after (10 sec) the footshock on day 1 to reveal the analgesic response to acute stress. Across the next 6 days, tail-flick latencies continued to be measured prior to the administration of footshock to assess the analgesic response to classical conditioning procedures. Each animal was sacrificed and the brain and spinal cord were removed for biochemical analysis of amine neurotransmitters. Although tail-flick latencies acutely-following footshock were significantly ($p < 0.05$) reduced in 6-OHDA-treated rats as compared to shocked controls, both LC ($p < 0.01$) and CM ($p < 0.05$) groups exhibited significant analgesic responses. Similar intermediate responses were observed to the classical conditioning procedures in the 6-OHDA-treated rats, with both groups of rats being significantly ($p < 0.01$) different from shocked and nonshocked controls. These data continue to emphasize a role of NE in mediating autoanalgesic phenomena. The intermediate effects of 6-OHDA treatment may reflect partial depletion of NE (currently being measured) or the involvement of additional neurochemical mediators.

232.4 SPINAL 5-HT RECEPTOR SUBTYPES AND NOCICEPTION. F.P. Zemlan, M.M. Behbehani, L.-M. Kow Pfaff. Departments of Psychiatry and Physiology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

The bulbospinal serotonin (5-HT) system is thought to suppress spinally organized nociceptive reflexes by raising nociceptive thresholds. The present study examined in the rat, four spinally organized nociceptive reflexes, freed from descending control due to spinal transection (T_{10}). Lumbar enlargement 5-HT receptors were stimulated by systemic administration of the centrally acting 5-HT agonists 5-MeO-dimethyltryptamine (0.1 to 2.0 mg/kg) and quipazine (5 to 20 mg/kg) during the initial two weeks after spinal transection. Unexpectedly, 5-HT agonists lowered nociceptive reflex thresholds and expanded reflex receptive field areas in a dose dependent manner. Both effects indicate a 5-HT induced facilitation of nociceptive input in the spinal preparation, rather than the 5-HT induced inhibition reported for the same reflexes in the intact animal. The facilitation of nociceptive spinal reflexes by centrally acting serotonin agonists observed in the present study, was blocked by the serotonin receptor blocker, metergoline (0.5 mg/kg), but not by the noradrenergic receptor blocker, phenoxybenzamine (20 mg/kg).

By postoperative Day 7 a clear shift to the left in the 5-HT agonist dose-response curves occurred indicating a functional supersensitivity. Receptor binding experiments investigated whether this 5-HT functional supersensitivity was correlated with a supersensitivity of spinal 5-HT_1 receptors labeled by ^{3}H-5-HT. A significant 37% increase in specific high affinity ^{3}H-5-HT binding was observed during the first week after transection. Scatchard analysis indicated that the increased ^{3}H-5-HT binding was due to an increased number of 5-HT_1 binding sites (Transected B_{max} = 199 + 19 f moles/mg, sham B_{max} = 107 + 15 f moles/mg), with no change observed in the apparent receptor affinity.

Literature is reviewed which suggests that the bulbospinal 5-HT system may differentially affect local spinal sensory input as opposed to ascending sensory input. The former system subserves local spinal nociceptive reflexes and is facilitated by the bulbospinal serotonin system while the latter relays nociceptive input centrally and is suppressed by the descending 5-HT system. Further, as the demonstrated functional and receptor supersensitivities were correlated, the present data suggest that the 5-HT facilitation of local spinal nociceptive reflexes is mediated by spinal 5-HT_1 receptors.

232.5 ANTAGONISM OF MICROSTIMULATION-PRODUCED ANALGESIA BY INTRATHECAL INJECTION OF METHYSERGIDE AND YOHIMBINE
N.M. Barbaro*, D.L. Hammond and H.L. Fields, Departments of Neurology, Physiology and Neurosurgery, University of California, San Francisco, CA 94143 and G.D. Searle & Co., Skokie, IL 60076.

Activation of bulbospinal serotonergic and noradrenergic fibers is postulated to mediate stimulation-produced analgesia (SPA) evoked from the nucleus raphe magnus (NRM) and nucleus reticularis paragigantocellularis (NRPG) respectively. Previous evaluation of this postulate has been hampered by the use of relatively high current intensities which do not permit anatomical resolution of effects produced by stimulation in these adjacent nuclei.

In the present study, Sprague-Dawley rats were acutely prepared with an intrathecal catheter and with two stimulating electrodes, one positioned in NRM, the other in NRPG. Baseline tail flick latencies (TFL) and paw pinch withdrawal thresholds (PWT) (Randall-Selitto test) were measured in these lightly anesthetized animals. The threshold current ($\leq$ 10 μA) required to evoke SPA was then determined for each site and test and then used throughout the experiment. Either the serotonergic antagonist methysergide (30 μg, 10 μl) or the noradrenergic antagonist yohimbine (30 μg, 10 μl) was then injected intrathecally. Fifteen minutes later, TFL and PWT were measured. NRM and NRPG were individually stimulated while TFL and PWT were measured. Then the other antagonist was added and the procedure was repeated. At completion of the study, the sites of stimulation and the position of the intrathecal catheter were verified histologically.

In agreement with previous work from this laboratory, threshold microstimulation in the NRM and NRPG produced a significant and quantal increase in TFL from a mean of 3.9 sec to above 10 sec (cut-off). Stimulation at the same sites also produced a significant and quantal increase in PWT from a mean of 216 g to above 800 g (cut-off). Intrathecal administration of either yohimbine or methysergide alone did not significantly antagonize the stimulation-produced increase in TFL or PWT evoked from either the NRM or NRPG. However, concomitant intrathecal administration of these two antagonists did antagonize the stimulation-produced increase in TFL and PWT from both nuclei. Intravenous administration of 30 μg of either drug or both was ineffective, as was intrathecal injection of saline.

These data indicate that both noradrenergic and serotonergic neurons projecting to the spinal cord mediate the descending inhibitory effects on nociceptive transmission exerted by NRM and NRPG in the rat.

Supported by PHS grant DA 01949, the National Migraine Foundation and G.D. Searle & Co.

232.6 FAILURE OF INTRATHECALLY ADMINISTERED THIP TO PRODUCE ANALGESIA.
D.L. Hammond and E.J. Drower*, Biological Research, G.D. Searle & Co., Skokie, IL 60077.

The analgesic properties of an isozaxole derivative of the GABA-mimetic muscimol, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP), have recently been reported in the rodent following systemic administration. However, the site of action remains unknown. In the present study, THIP was administered intrathecally to determine if its analgesic activity was mediated by an effect exerted at the level of the spinal cord. Male Sprague-Dawley rats were prepared with an indwelling catheter in the spinal cord subarachnoid space. Seven days later, the animals' responsiveness to noxious thermal stimuli was determined using the tail flick and hot plate tests. After baseline tail flick latencies (TFL) and hot plate latencies (HPL) had been determined, the rats were injected intrathecally with either THIP (1-50 μg), muscimol (0.25-1 μg), or baclofen (1-10 μg). TFL and HPL were measured 10, 30 and 60 min later. Within 10 min of the intrathecal injection of THIP (5-50 μg), a marked dose-dependent flaccidity of the hindlimbs and tail occurred. The animals could not be tested on the tail flick or hot plate; however, they continued to vocalize to noxious pinch of the extremities. Intrathecal injection of lower doses of THIP that did not produce flaccidity (1-2 μg), did not increase TFL or HPL, or affect responsiveness to noxious pinch. Similarly, muscimol (0.5-1.0 μg) produced motor impairment and did not alter the animals' response to noxious pinch. Intrathecal injection of a lower dose that was without motor effects (0.25 μg) significantly increased TFL (3.3 $\pm$ 0.2 sec to 7.5 $\pm$ 1.6 sec), but did not alter HPL or decrease the response to noxious pinch. For comparison purposes, baclofen was also injected intrathecally. While 10 μg of baclofen produced motor incoordination, it also decreased responsiveness to noxious pinch. Intrathecal injection of 1 μg of baclofen did not produce motor impairment and significantly increased both TFL (2.5 $\pm$ 0.2 sec to 7.5 $\pm$ 1.8 sec) and HPL (7.8 $\pm$ 1.1 sec to 19.8 $\pm$ 5.3 sec), as reported previously. These data indicate that the site at which the GABA-mimetic THIP acts to produce analgesia does not involve the spinal cord.

232.7 RELEASE OF ENDOGENOUS NOREPINEPHRINE AND SEROTONIN INTO SPINAL CORD SUPERFUSATES FOLLOWING LOCAL INJECTION OF PHENTOLAMINE INTO THE NUCLEUS RAPHE MAGNUS.
H.K. Proudfit and J. Sagen. Dept. of Pharmacology, Univ. of Illinois at Chicago, Chicago, IL 60680.

Activation of neurons in the nucleus raphe magnus (NRM) produces hypoalgesia which most likely results from inhibition of spinal cord pain transmission pathways. Previous reports from this laboratory have demonstrated that blockade of the noradrenergic (NA) input to the NRM by microinjecting alpha-adrenergic antagonists produces hypoalgesia. These studies suggest that NA neurons inhibit the activity of NRM neurons. Subsequent studies have demonstrated that this hypoalgesia can be blocked by intrathecal injection of adrenergic or serotonergic antagonists as well as by depletion of spinal cord norepinephrine (NE) or serotonin (5-HT). These studies suggest that the hypoalgesia produced by injection of NA antagonists in the NRM is mediated by both bulbospinal NA neurons and raphe-spinal serotonergic neurons. The present studies were designed to directly measure the release of endogenous NE and 5-HT from the spinal cord following injection of the NA antagonist phentolamine (PTL) into the NRM.

Female rats (350 g) were anesthetized with urethane and fitted with inflow and outflow tubes for spinal cord superfusion. Three control samples, three ml each per 30 min period, were collected and then PTL was injected into the NRM. Three 30-minute samples were collected following PTL injection. The amount of NE and 5-HT released into the superfusates was determined by isolating the amines on a CG-50 column, lyophilizing the eluate, and quantitating the amount in each sample using HPLC and electrochemical detection. The injection site in each animal was verified histologically.

The average basal release of NE and 5-HT before the injection of PTL in the NRM was 0.27 +/- 0.04 and 0.31 +/- 0.11 ng/ml per 30 min., respectively. Within 30 min after the injection of PTL the NE concentration rose to 0.56 +/- 0.12 ng/ml and that of 5-HT rose to 0.80 +/- 0.32 ng/ml. The elevated amine concentrations in the superfusates returned to basal levels by 90 min. In contrast the injection of saline into the NRM did not affect the concentrations of NE or 5-HT in the superfusates. These studies support our proposal that the local injection of PTL into the NRM produces hypoalgesia which is mediated by the release of both 5-HT and NE in the spinal cord. (This work was supported by USPHS Grant NS 18636 and the PMA Foundation)

232.8 EVIDENCE FOR THE INVOLVEMENT OF DESCENDING NORADRENERGIC PATHWAYS IN THE ANALGESIC ACTION OF BACLOFEN.
J. Sawynok and C. Dickson*, Dept. of Pharmacology, Dalhousie Univ., Halifax, N. S., Canada, B3H 4H7

Noradrenergic systems originating in the brainstem appear to exert an inhibitory effect on the transmission of nociceptive information in the spinal cord (Fed. Proc. 40, 1981, p.278). The role of descending noradrenergic pathways in the analgesic action of baclofen was investigated because (a) microinjection of baclofen into lateral brainstem sites produces analgesia (Eur. J. Pharmacol. 57, 1979, p. 43) and (b) spinal transection reduces analgesia produced by systemic baclofen (Eur. J. Pharmacol. 47, 1978, p.159). Neurotoxins and receptor antagonists were administered intrathecally (i.t.) to rats implanted with chronic indwelling catheters, while baclofen was injected intraperitoneally (i.p.). Analgesia was assessed using the tail flick (baseline latency 2-3 sec) and hot plate (50°C) methods. 6-Hydroxydopamine (6-OHDA, 20 μg) significantly inhibited analagesia produced by baclofen, reducing the analgesic index (sum of latency minus baseline for 4 readings at 30 minute intervals following injection of baclofen) to 30-50% of control values in both tests 4, 7 and 13 days following i.t. administration. A higher dose (50 μg) injected intraspinally (i.s.) reduced the index to approximately 20% of control values 7 days after injection. 5,6-Dihydroxytryptamine (5,6-DHT, 20 and 100 μg) did not reduce analgesia, but increased it slightly in the tail flick test within two weeks of injection. Both 6-OHDA and 5,6-DHT produced hyperalgesia in the tail flick test during these time intervals when baseline latencies were increased to 6-8 seconds, an effect less reliably observed with the lower baseline latency and with the hot plate test. In other experiments, baclofen was injected i.p. while the alpha antagonists phentolamine and tolazoline were injected, i.t. 40 minutes later when the analgesic effect of baclofen was well developed. Phentolamine, 50 and 100 μg (but not 30 μg), produced a dose-related reduction in analgesia in the tail flick test, the higher dose reducing latencies to near baseline values. A similar reduction in analgesia was observed with tolazoline (100 μg). Phentolamine alone (50 and 100 μg) produced hyperalgesia when 6-8 second baseline tail flick latencies were used. These results suggest that a major component of the analgesic effect of systemically administered baclofen is mediated by descending noradrenergic pathways and subsequent activation of alpha receptors in the spinal cord. (Supported by MRC Canada).

232.9 INTRATHECAL SUBSTANCE P AND RELATED PEPTIDES:INHIBITION OF MORPHINE ANALGESIA AND HYPERALGESIA. S.M. Moochhala* and J. Sawynok, (SPON: F. S. LaBella), Dept. of Pharmacology, Dalhousie University, Halifax, N. S. Canada, B3H 4H7

Morphine (M) can produce analgesia by a local action in the spinal cord (Science 192, 1976, p. 1357). Substance P (SP) has been proposed as a primary afferent transmitter of nociceptive information, and the demonstrated ability of M to inhibit its release in vitro (Nature 268, 1977, p. 549) and in vivo (Nature 286, 1980, p. 155) may contribute to this spinal analgesic action. The objectives of this study were (a) to determine whether SP could inhibit the spinal analgesic action of M and to characterize the receptor subtype involved by comparing the effects of physalaemin (P) and eledoisin related peptide (ERP) to those of SP, and (b) to further examine the hyperalgesic effect of SP by determining dose-response characteristics, comparing the effects of P and ERP to those of SP and determining whether desensitization and cross-desensitization would occur. All drugs were administered intrathecally (i.t.) to rats implanted with chronic indwelling catheters. Analgesia was assessed using the tail flick (baseline latency 2-3 sec for interactions with M and 6-8 sec for hyperalgesia experiments) and hot plate (50°C) methods. Morphine (1.5-3.5 µg) produced dose-related analgesia in both tests. SP (5-15 µg) inhibited analgesia produced by 2.5 µg M in a dose-related manner, reducing the analgesic index (sum of latency minus baseline for 4 readings at 15 min intervals) to 60-70%, 35-50% and 10-20% of control values at 5 µg (4 nmole), 10 µg (7 nmole) and 15 µg (11 nmole) respectively. ERP (approx. 25 nmole) and P (approx. 5 nmole) reduced the analgesic index to 40-60% of control values, indicating a rank order of potency of P ≥ SP > ERP. In other experiments, SP (1.9-11 nmole) produced dose-related hyperalgesia as well as scratching and grooming behaviour. Both P and ERP also produced hyperalgesia with a rank order of potency similar to that observed previously. Desensitization to the hyperalgesic effect of SP was produced by 3 consecutive doses of 15 µg (11 nmole) SP. P and ERP did not produce hyperalgesia when rats were made insensitive to SP, suggesting an action on the same receptor. The observed inhibition of M analgesia by SP (via a physalaemin-type receptor) is consistent with the notion that this action may be due to an inhibition of SP release. However, less than specific effects may be involved because SP also inhibits the spinal analgesic action of noradrenaline and baclofen. (Supported by MRC Canada).

232.10 EVIDENCE FOR A CHOLECYSTOKININ (CCK)-LIKE ENDOGENOUS OPIATE ANTAGONIST. I.B. Kinscheck*, L.R. Watkins, E. Kaufman & D.J. Mayer (SPON: A. Szumski). Dept. Physiol. Biophys., Med. Coll. Va./Va. Commonwealth Univ., Richmond, VA 23298.

Exogenous CCK specifically antagonizes opiate analgesia via CCK/gastrin receptors, suggesting a physiological role for CCK in pain processing. The introduction of the specific CCK/gastrin antagonist, proglumide (PR, gift of A.H. Robins), provides a direct means of determining whether an endogenous CCK-like opiate antagonist exists. We therefore tested the effect of intrathecal (IT) PR on various opiate & nonopiate analgesias in rats as assessed by the tail flick (TF) test. Parameters were chosen so that each analgesic manipulation produced submaximal analgesia. IT PR alone (0, 0.02, 0.2, 0.6 µg) did not produce analgesia through 95 min after injection. A biphasic dose response curve was seen in response to 5 doses of IT PR tested against 1 µg IT morphine. Maximal potentiation was produced by .01 µg PR, which hastened the onset of analgesia without affecting peak analgesia, while 5 µg markedly attenuated analgesia through 95 min post-injection. The maximal potentiating dose, .01 µg, was subsequently tested against other analgesias. The opiate analgesias produced by periaqueductal gray morphine (3 µg) and by front paw shock (90 s, 1.6 mA rms) were also both significantly potentiated by this IT PR dose. Two independent forms of nonopiate analgesia, 10 µg IT norepinephrine bitartrate and 90 s, 1.6 mA rms hind paw shock of spinalized animals, were tested to assess specificity. Both analgesias were attenuated by .01 µg IT PR.

Thus CCK/gastrin appears to function as a specific opiate antagonist which is activated in response to acute opiate administration or release. As CCK does not bind to opiate receptors, a CCK receptor-opiate receptor interaction may occur which causes a decrease in opiate analgesic efficacy. Such a dynamic interaction would allow the animal to maintain nociceptive responsiveness via an opponent process mechanism.

We also tested whether this endogenous antagonist could play a role in the phenomenon of opiate tolerance. Rats were made tolerant via either 3 or 6 daily IT injections of 10 µg morphine & subsequently challenged with 5 µg IT morphine. Neither PR (1.8 µg) in the absence of morphine nor 5 µg morphine in the absence of PR produced analgesia. Animals receiving both PR & 5 µg morphine demonstrated analgesia in a dose-dependent manner. Animals exposed to the longer tolerance regimen required a higher dose of PR to induce analgesia. Maximal analgesia in these rats was produced by 1.8 µg PR plus 5 µg morphine. Thus CCK may be involved in a negative feedback system which attempts to maintain optimal nocisponsiveness, thus producing "tolerance." Supported by PHS grant DA 00576 to DJM.

232.11 CENTRAL NEUROTRANSMITTERS MODIFICATIONS AFTER EPIDURAL CORD STIMULATION IN THE RAT. E.A. Parati, G. Broggi*, B. Regi*, A. Franzini*, D. Servello*and M. Parenti. Neurological Inst. "C. Besta" and Dept. Pharmacology, Univ. of Milan, Sch. of Med., Milan, Italy.

The electrical stimulation of the dorsal cord (DCS) has been proved to be effective in the treatment of human incurable pain. However, the mechanisms underlying the therapeutic action of DCS are still unknown. In order to investigate the neurochemical correlates of DCS we used the rat as an experimental animal model. Monopolar electrodes were inserted epidurally at the level of the thoracic portion of the cord. Twenty four hours later, the animals, maintained freely moving in their cages, were stimulated electrically continuously for 6 hours at 1,000 Hz (the electrical parameters used were 1 mA, 1.5 Volts and 1 msec). At the end of this session the animals were killed and the cerebellum, brainstem, striatum and spinal cord removed for neurochemical measurements. Sham-operated animals were used as controls. The concentrations of serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine and norepinephrine were then assayed using HPLC with electrochemical detection. While no significant alterations were detected in the catecholamine content of the different areas, a marked change in the indoleamine levels was found in the spinal cord, cerebellum and brainstem (see table). These results seem to be promising in view of the involvement of serotonergic system in pain modulation. Further studies are in progress to evaluate if opioid peptides and Substance P are altered after DCS.

	5-HT (ng/g)		5-HIAA (ng/g)	
	CONTROLS	STIMULATED	CONTROLS	STIMULATED
Spinal cord	272.3±4.3	267.1±5.5	122.8±6.1	178.9±12.1**
Cerebellum	54.1±2.3	73.2±4.6**	16.1±3.9	27.2± 7.4
Brainstem	333.8±3.7	451.2±53.9*	326.4±13.2	501.4±82.9*
Striatum	660.6±49.0	768.4±75.6	401.1±35.3	574.1±148.0

*$p < 0.05$

**$p < 0.01$

Each value refers to the mean ± S.E.M. of at least ten determinations.

232.12 THE RAT SPINAL CORD MET-ENKEPHALIN INCREASE, INDUCED BY LOCALIZED PAIN, IS MEDIATED BY AFFERENT FIBERS. M. Trabucchi, E. Faccini*, H. Uzumaki*, G. Pasinetti*, S. Govoni. Dept. of Pharmacology, University of Brescia, Italy.

Pharmacological and electrophysiological evidence indicates that endogenous opiates modulate pain transmission at spinal cord level. Met-enkephalin interneurons might represent the putative spinal gating control system and be regulated by a complex polysynaptic mechanism. In order to demonstrate whether met-enkephalin neuronal function is related to the activity of primary afferent fibers and thus to the incoming painful stimuli, the concentration of this endogenous opiate was measured in the cervical, thoracical and lumbar segments of the spinal cord after the injection of Freund's adjuvant either in the fore or in the hind paws of control or denervated rats. The results indicate that chronic localized pain induces a selective increase (+ 50%) in met-enkephalin immunoreactive material in the dorsal horn of the spinal cord segment which receives a direct projection from the inflamed paw. Denervation obtained by means of the sectioning of the plexus brachialis or of the sciatic nerve before inducing inflammation prevents the increase of spinal cord met-enkephalin content. In the same experimental conditions the injection of Freund's adjuvant in a paw significantly reduces the vocalization threshold in the paw pinch test. Moreover, denervated limbs were completely insensitive to any stimuli, although after Freund's adjuvant injection the degree of inflammation was comparable with that of controls. The data presented confirm the hypothesis that chronic pain induces a change in met-enkephalin content which is selective for the spinal cord segment receiving afferent fibers, the integrity of which is necessary for the activation of the spinal gating system made up of met-enkephalin interneurons.

232.13 AN AUTORADIOGRAPHIC DEOXYGLUCOSE STUDY OF SOME ACUPUNCTURE-PRODUCED METABOLIC CHANGES IN THE CENTRAL NERVOUS SYSTEM OF THE RAT. Peter J. Hand, Shyh-Chang Sheu* and Hongchien Ha*. Dept. of Animal Bio., Sch. of Vet. Med., Inst. of Neurol. Sci., Univ. of Penna., Phila., PA 19104 and Nat. Yang-Ming Med. Coll., Taiwan, R.O.C.

The quantitative (^{14}C) deoxyglucose (2DG) method was employed to study some central effects of acupuncture. 8 unanesthetized, restrained rats were pulse injected with 2DG and received either (1) intermittent noxious thermal stimulation (56°C water applied to the tail for 4 secs every 30 sec) during the 45 minute post injection period (N=1), (2) the same noxious stimulus and unilateral acupuncture of tsu-san-li point (proximal anterior tibial muscle) for 45 minutes (efficacy of acupuncture was determined by measuring tail flick response latencies=419-61%) (N=3), (3) unilateral tsu-san-li stimulation only for 45 minutes (N=3), or (4) non-noxious (40°C) thermal stimulation of the tail for 45 minutes (control; N=1). All rats were then prepared according to Sokoloff (J. Neurochem., 1977). Following noxious stimulation, a number of structures exhibited substantial increases (24-44% above control values) in local cerebral metabolic rates of glucose (LCMRG): medial medullary reticular formation; rostral medullary raphe, midbrain central gray and dorsal raphe; n. cuneiformis; n. reunie ns-central medianus complex of thalamus; intralaminar nuclei of thalamus; dorsomedial, rostromedial, dorsolateral, and lateral thalamus; and neocortex (particularly parietal areas). Following tsu-san-li stimulation alone, and with the exceptions of midbrain central gray and medullary raphe (LCMRG 26% above control values), the above structures exhibited small to moderate increases (7-19% above control values) in LCMRG. When noxious thermal and tsu-san-li stimuli were paired, LCMRG in the above regions either exhibited small increases (3-12%), no change (medullary reticular formation, n. reuniens-central medianus complex), or a small (3-7%) decrease (rostromedial, submedius nuclei of the thalamus) when compared to control values. Lower spinal cord segments are in the process of being examined in all of the above animals.

In conclusion, the data indicate that: (1) noxious thermal stimulation of the tail produces significant increases in 2DG labeling of a number of structures, some of which have been implicated in the conduction of noxious information and in the production of analgesia, (2) tsu-san-li stimulation alone produces moderately increased 2DG labeling of a number of the same brain structures examined in (1), and (3) the pairing of tsu-san-li and noxious thermal stimulation of the tail results in an interaction, which produces significantly less brain labeling than was present following noxious or acupuncture stimulation alone. Additional studies to determine the mechanisms of such an interaction are in progress. (Supported by grant NS-14935 and National Yang-Ming Medical College).

232.14 EFFECTS OF ELECTRO-ACUPUNCTURE AND NALOXONE ON CUTANEOUS PAIN THRESHOLDS AND PLASMA CONCENTRATIONS OF β-ENDORPHIN (β-EP) IMMUNOREACTIVITY IN SHEEP. D. F. B. Bossut*, M. W. Stromberg, and P. V. Malven. Departments of Vet. Anat. and Anim. Sci., Purdue University, West Lafayette, IN 47907.

Analgetic effectiveness of electro-acupuncture (EA) treatment and plasma concentrations of β-EP were measured in 26 male and female sheep. Cutaneous pain thresholds (PT) were quantified by scoring (0 to 9) the animal's reaction to cutaneous stimuli (pinprick, pinch, and contact heat) in the following 7 areas of the body: head, neck, thorax, forelimb, ventral abdomen, flank, and hindlimb. Control trials consisted of measuring PT and drawing blood samples every 8-10 min, but without any needle insertions. For EA trials, acupuncture needles were inserted in specific lumbosacral points (denoted YAO PANG in Chinese) or in specific points in one forelimb (denoted SAN YANG LU in Chinese). Stimulating voltage and frequency (Chinese-made stimulator SB-71-2) were increased gradually to levels just below those at which the animal reacted adversely and these settings were maintained throughout the remainder of the trial. EA treatment of both loci increased the cutaneous PT ($P<.01$) in all 7 body areas (i.e. EA caused cutaneous analgesia). The elevated PT following EA treatment of YAO PANG but not of SAN YANG LU, was partially but significantly ($P<.05$) antagonized by exogenous naloxone (1.1 mg/kg). Plasma concentrations of β-EP measured by radioimmunoassay were not increased by measurement of PT during control trials (remained between 0.3 and 0.4 ng/ml). Plasma β-EP increased significantly ($P<.05$) during EA treatment reaching a maximum after 15-30 min of EA stimulation. When plasma β-EP was analyzed on the basis of analgetic outcome of each trial, β-EP concentrations in trials with poor analgetic outcome were not increased over those concentrations during control procedures. In contrast, β-EP concentrations in trials with good analgetic outcome were increased 2- to 3-fold (0.7 to 0.9 ng/ml). Increases in β-EP during EA treatment of SAN YANG LU locus were slightly greater and of longer duration than those increases following YAO PANG stimulation. Plasma β-EP declined from peak levels even though EA treatment continued. In summary these results have demonstrated a positive correlation between EA-induced cutaneous analgesia and increases in plasma β-EP levels. However, naloxone-induced antagonism of analgesia resulting from YAO PANG stimulation, but not SAN YANG LU stimulation, when both showed a positive correlation between analgesia and β-EP is difficult to interpret. Although this finding raises some doubt about a cause-effect relationship between β-EP and cutaneous analgesia, the ineffectiveness of the present dosage of naloxone with SAN YANG LU stimulation might be due to the greater activation of β-EP during EA of that locus. Supported in part by grant DA 2661.

232.15 DIFFERENTIAL EFFECTS OF NALOXONE ON ANALGESIA AND VOCALIZATIONS INDUCED BY BRIEF DURATIONS OF PULSED FOOTSHOCK. J. Timothy Cannon, Nicholas Maro*, Bartholomew Telep* and Gerald Carty*. Dept. of Psychology, University of Scranton, Scranton, PA 18510.

It has recently been found that subjecting rats to relatively brief continuous footshock (all four paws) can result in opioid or non-opioid forms of stress-induced analgesia (Terman, Lewis and Liebeskind, this volume and personal communication). Differential activation of opioid or non-opioid mechanisms could be effected by varying the duration of shock exposure and/or the shock intensity.

The present research attempted to determine whether the pulsed shock pattern used by Lewis, Cannon and Liebeskind (1980) to produce an opioid form of analgesia after prolonged (20-30 min) stress could produce both the opioid and non-opioid effects that Terman et al. observed using brief exposures (4 min or less) to continuous shock. An additional advantage of having pulsed shock delivery was the potential for recording shock-elicited vocalizations during and between(vocalization after-discharge) shocks. Given the anatomical and pharmacological distinctions that have been demonstrated for these forms of vocalizations, it was hoped that the ability to observe such behaviors during the stress exposures might advance our understanding of the underlying mechanisms of stress-induced analgesia.

The subjects (adult, male, albino rats) were run during the dark phase of their 12-12 hr light-dark cycle. In three separate experiments the animals were exposed to either 2.2, 3 or 3.5 mA of scrambled footshock (1 sec pulse every 5 sec). Within each experiment, animals were divided into two groups (10 animals each) that received 1 cc/kg S.C. injections of either saline or naloxone (3 mg/kg) as indicated below. Each animal was exposed to 2 and 4 min of footshock in separate sessions one week apart. The animals were injected 5 min prior to baseline tail-flick testing (1 trial/min for 5 min). Footshock was begun 1-3 min thereafter. Tail-flick latencies were again recorded over a 15 min post-stress interval. The observers were blind in relation to the type of drug injection. Analgesia rarely lasted beyond 7 min post-stress, consequently, only this time interval was statistically analyzed using the .05 level of significance.

Our results are in general agreement with those of Terman et al., in that they suggest that manipulations of intensity and duration can result in either opioid or non-opioid forms of analgesia after brief footshock stress. Significant post-stress elevations of tail-flick latencies occured at all shock levels. Naloxone significantly reduced this effect only in animals that received 3 mA for 4 min. Naloxone also significantly increased vocalization after-discharges only in animals exposed to 3.5 mA of shock.

232.16 SUPRASPINAL OPIOID ANALGESIA IS PRODUCED BY LATERAL HYPOTHALAMIC STIMULATION. S. Uysal* and K. Carr. Dept. of Psychiatry, New York Univ. Med. Ctr., New York 10016.

Previous work has shown that stimulation in the lateral hypothalamus (LH) ameliorates supraspinally-elicited aversion (K. Carr and E. Coons, Science, 215:1516, 1982). The present study examined whether LH stimulation alters responses to peripheral pain stimuli applied via intracutaneous tail electrodes. A pain test developed by Carroll and Lim (Arch. int. Pharmacodyn., 125:383, 1960) allowing measurement of responses that reflect activity at different levels of the CNS was employed.

Rats were tested in a small chamber with a tail-stock at the rear. A test session consisted of four series of tail shocks. Each shock was a 1-sec train of 1.6 msec pulses at 125/sec. Shocks were separated by a 30-sec interval and voltage was increased in steps of 0.25V until thresholds were obtained for eliciting (1) forelimb locomotor movement, (2) vocalization, and (3) vocalization afterdischarge. On alternate series, LH stimulation was delivered concurrently with tail stimulation. LH stimulation was a 1-sec train of 0.1 msec pulses at 25/sec. An LH stimulation intensity that in preliminary testing elicited 1/3 the maximum rate of self-stimulation was used because such intensities produced asymptotic ameliorative effects in the studies of supraspinally-elicited aversion.

In the six rats tested, LH stimulation had no effect on threshold for the locomotor response (mean change: +8% ±7.9) or vocalization (+29% ±9.4). LH stimulation did elevate threshold for vocalization afterdischarge (+62% ±10.6; $p<.01$). Afterdischarge has been shown in transection studies to be organized at a higher level of the neuraxis than the other two responses and appears to reflect the "affective" dimension of pain. These results therefore support previous evidence that LH stimulation preferentially interacts with pain mechanisms at a supraspinal level to diminish the affective consequences of intense somatic stimulation.

When rats were injected with naltrexone (10.0 mg/kg, s.c.) 20 min prior to testing, the LH stimulation-produced elevation of threshold for vocalization afterdischarge was blocked ($p<.01$). This dose of naltrexone had no effect, in a later test, on intensity thresholds for eliciting self-stimulation through the same LH electrodes.

These results indicate that stimulation in the LH activates an opioid analgesic process which preferentially inhibits supraspinal activity associated with the affective dimension of pain. Moreover, as is true of the LH stimulation-produced amelioration of supraspinally-elicited aversion, this effect is dissociable from the self-stimulation reward process.
(Supported by NIH MH 35976 and NIH BRSG S07 RR05399-21)

232.17 MORPHINE-INDUCED SUPPRESSION OF THE JAW-OPENING REFLEX IN RABBITS: A STUDY OF ELECTRICALLY STIMULATED DENTAL PULP PAIN. A.L. Curtis* and W.J. Anderson. Dept. Life Sciences, Indiana State Univ., Terre Haute, IN 47809 and Indiana Univ. Sch. Med., Terre Haute Ctr. Med. Ed., Terre Haute, IN 47809.

The purpose of this study was to clarify the electrical stimulus parameters and dose range requirements of the analgesic agent morphine sulphate (MOR) in order to obtain an effective suppression of the jaw-opening reflex in rabbits. The delineation of these conditions have indicated a differential morphine sensitivity in the trigeminal system. Statistically significant and similar response/time plots were obtained from the mandibular left incisor pulp stimulated at a high intensity stimulus (strength: four times the threshold for the response; frequency: twin pulse 0.1 ms duration, 0.8 ms delay at 1 Hz) over a dose range of 3, 6, and 12 mg/kg MOR as compared to the maxillary left incisor pulp stimulated at a low intensity stimulus (strength: three times the threshold for the response; frequency: single pulse 0.1 ms duration at 1 Hz) over a 6, 12, and 24 mg/kg MOR dose range. The pain stimulus was the electrical stimulation of the dental pulp. The pain response was the evoked jaw-opening reflex recorded from the left digastric muscle and indexed by the respectively averaged peak to peak measure of the electromyogram (EMG) and its latency (LAT). All morphine was administered via cannulated left carotid artery. The significant MOR-induced effects upon the pain response was the decrease of the EMG and the increase of the LAT, both of these divergencies being naloxone reversible. However, the high intensity stimulus applied to the maxillary left incisor pulp resulted in response/time plots that possessed no significant difference from control, even over a 6, 12, and 24 mg/kg MOR dose range. There is evidence of anatomical differences, beyond those dictated by somatotopy, between maxillary and mandibular dental pulp central projections in the spinal trigeminal nucleus. One such report by Westrum et al. (Exp. Neurol., 74: 787, 1981) indicates a greater number of horseradish peroxidase-labeled fibers and terminals for the maxillary canine over the mandibular canine in their cats as well as differences in their laminar distributions in rostral pars caudalis suggesting different synaptic input patterns. In concert with these anatomical differences, this study offers evidence of a physiological difference in the trigeminal system.

232.18 VOCALIZATIONS AS MEASURES OF PAIN AND HYPALGESIA IN THE STUMPTAIL MACAQUE. Cooper, Brian Y. and Vierck, C. J. Jr., Dept. of Neuroscience, Univ. of Florida, Gainesville, FL, 32610.

Stumptail macaques (macaca Speciosa), when trained to pull a bar to escape painful electrical stimulation, emit a large number of vocalizations. These vocalizations occur in reliable patterns, clustering 0-15 seconds following the shock-escape trial (the reactive period), but also appearing irregularly during the remainder of the intertrial interval (the waiting period). Spectrographic analysis of these vocalizations revealed that the predominant form of vocalization was the 'bridge scream' (Bertrand, 1965), which made up 80% of the total vocalization content. Other vocalizations, including grunts, whines and squeaks were observed, but these made up smaller percentages of the overall content and were highly individualized. Bridge screams were emitted most often in the reactive period but occurred throughout the intertrial intervals and were not related in frequency, duration, or amplitude to the intensity of preceeding trials.

Monkeys trained to pull a bar to receive a food reward also emit bridge screams. These vocalizations occur in similar patterns as for monkeys receiving electric shock. Also, following an extensive period of exposure to non-painful shock conditions (six weeks), monkeys previously exposed to painful shock continued to vocalize in similar patterns. Therefore, vocalizations in the macaque were not uniquely associated with pain in the experimental situation. Nevertheless, they were modulated by analgesics and other compounds with putative modulatory influences on pain sensations.

Following treatment with morphine (.25-3 mg/kg), the frequency of vocalizations rapidly decreased during the reactive and waiting periods. With small doses of morphine (.25-1 mg/kg), vocalization amplitudes typically decreased except during the immediate post-shock period. There was little evidence of changes in vocalization content (types of call) by morphine.

Attempts to block or enhance the effects of morphine by depletion of serotonin or noradrenaline produced effects that paralleled those found with measures of operant reactivity (bar pull force and latency of escape; Cooper & Vierck, 1980; Vierck, Cooper, Franzen, Ritz & Greenspan, 1983) - that is, weak blockade with PCPA and weak enhancement with Disulfiram. However, the inability to demonstrate that the frequency, intensity, duration, or content of vocalizations were reliably related to the intensity of painful stimuli suggests that these monoamine effects do not represent modulation of pain sensitivity or mediation of opiate hypalgesia.

232.19 LATERALITY OF ANALGESIA PRODUCED BY INTRAVENTRICULAR MORPHINE IN THE RAT. S.R. Cohen, F.V. Abbott, and R. Melzack*. Dept. of Psychology, McGill University, Montréal, P.Q., Canada.

The analgesic effect of morphine injected into the lateral ventricle of the rat was studied in two pain tests: the formalin test which assesses the behavioral response to tonic (continuous) pain generated in injured tissue, and the foot-flick test which examines phasic (brief) threshold-level pain. In the formalin test, morphine injected into the lateral ventricle produced analgesia when the ipsilateral hindpaw was injured but had no analgesic effect when the contralateral hindpaw was injured. In contrast, intraventricular morphine produced analgesia bilaterally in the foot-flick test. Analgesia was achieved with relatively low doses of morphine (2.5-10.0 μg) in the formalin test while very high doses (50-200 μg) were necessary to produce analgesia in the foot-flick test. In the formalin test, morphine's effect was as large during the first time block of testing (11-20 min after intraventricular injection) as at any other time. In the foot-flick test, morphine's effect reached a maximum at 30-45 min after intraventricular injection. These results add to other data indicating that different neural mechanisms underlie opiate analgesia in different types of pain. Moreover, they indicate that, in a test of tonic pain, the neural mechanisms of morphine analgesia are somatotopically organized and that forebrain structures are likely to be involved.

232.20 EFFECTS OF CONCOMITANT ADMINISTRATION OF MORPHINE AND d-AMPHETAMINE ON ESCAPE BEHAVIOR MAINTAINED BY AVERSIVE INTRACRANIAL STIMULATION. S. Sasson, E.M. Unterwald* and C. Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Several clinical reports suggest that the concomitant administration of morphine and d-amphetamine may be useful in the management of pain (e.g., Forrest et al., New Engl. J. Med., 296:712-715, 1977). We have found that morphine as well as other opioid analgesics will raise the threshold for escape from aversive electrical stimulation to the mesencephalic reticular formation (MRF) of the rat. The present study was conducted to determine if d-amphetamine would potentiate the analgesic action of morphine in this pain model.

Male albino rats (CDF - Charles River Laboratories) were stereotaxically implanted with bipolar stainless steel electrodes aimed at the MRF. Following recovery from surgery, animals were trained to turn a wheel manipulandum to escape from aversive electrical stimulation to this midbrain site. The threshold for escape was determined by varying the current intensity according to a modification of the psychophysical method of limits. d-Amphetamine (0.06-4.0 mg/kg, i.p.) had no effect or slightly lowered the escape threshold, depending on the animal. Morphine (1.0-16.0 mg/kg, i.p.), as in previous studies, caused a dose-related increase of the threshold. A dose of morphine, which by itself did not significantly raise the escape threshold, was then studied with concomitant administration of various doses of d-amphetamine.

This drug combination caused significant increases in the escape threshold which were directly related to the d-amphetamine dose. Recent work in our laboratory suggests that this effect is not due to actions on attentional or perceptual systems but are due to direct effects on a pain system. These data suggest the involvement of midbrain structures in the mediation of this drug effect and support findings from clinical studies which show that d-amphetamine potentiates the analgesic action of morphine.

(Supported in part by NIDA grant DA 02326).

232.21 OPIOID AND NONOPIOID FORMS OF STRESS-INDUCED ANALGESIA IN THE MOUSE: STRAIN COMPARISONS. A.S. Moskowitz, G.W. Terman and J.C. Liebeskind. Psychology Dept., UCLA, Los Angeles, CA 90024.

Recent work in rats has demonstrated that inescapable footshock elicits either opioid- or nonopioid-mediated analgesia depending on the shock parameters (Lewis et al., Science, 208: 623, 1980). Prolonged, intermittent footshock induces an opioid-mediated analgesia, whereas brief, continuous footshock induces analgesia not mediated by opioids.

In this study we compared opioid and nonopioid forms of stress-induced analgesia in two strains of female mice, C57BL/6BY and CXBK (Jackson Labs). The CXBK strain has been reported to have a lower number of whole brain opiate binding sites and is less sensitive to the analgesic effect of morphine than the C57 strain (Baran et al., Life Sci., 17: 633, 1975). Inescapable footshock (60-Hz sine waves) was delivered through a scrambler to a grid floor. The parameters necessary to induce the opioid and nonopioid forms of stress analgesia were ascertained using the criterion of naltrexone blockade (5 mg/kg, s.c.). Pain responsiveness was measured by the tail-flick method.

Opioid-mediated analgesia was induced by prolonged (10 min, 3.5 mA), intermittent (1 sec on, 4 sec off) footshock, whereas brief (4 min, 1.0 mA), continuous footshock was found to elicit a nonopioid form of stress analgesia. The CXBK strain showed significantly ($p < 0.05$) less of the opioid form of stress analgesia than the C57 strain, whereas the two strains did not differ in regard to nonopioid stress analgesia. Using in vitro autoradiography, the CXBK strain is also found to have a lower number of opiate binding sites than the C57 strain in brain and spinal cord areas associated with pain processing (Goodman et al., Soc. Neurosci. Abs., 8: 221, 1982). These findings support the hypothesis that separate opioid and nonopioid pain inhibitory systems exist and indicate that genetic factors influence the analgesic response to stress. (Supported by NIH grant NS07628 and by a gift from the Brotman Foundation. A.S.M. was supported by Mental Health Training Grant MH 15345.)

232.22 THE ROLE OF CURRENT INTENSITY IN OPIOID AND NONOPIOID STRESS-INDUCED ANALGESIA. G.W. Terman and J.C. Liebeskind. Dept. of Psychology, UCLA, Los Angeles, CA 90024.

The finding that exposure to certain forms of stress can produce potent analgesia has generated much research on the neurochemical and neuroanatomical substrates of this phenomenon. Such stress-induced analgesia has been shown to be multi-faceted, depending in some instances on opioid peptides and in others on extra-opioid mechanisms. The critical variables responsible for differential production of opioid- and nonopioid- stress analgesia have been investigated in several laboratories. We have demonstrated that the opioid or nonopioid nature of analgesia inescapable footshock depends on the temporal parameters of its administration. More specifically, whereas 1 or 2 min of continuous footshock produces analgesia sensitive to naltrexone antagonism (opioid), 3, 4 or 5 min of continuous footshock at the same intensity produces an equipotent analgesia not blocked by this drug (nonopioid). Others have reported a differential production of opioid and nonopioid stress analgesia as a function of body region shocked and number of shocks administered. We now show the importance of current intensity in determining the opioid or nonopioid nature of stress analgesia.

Eighty male Sprague-Dawley rats were assigned to one of ten groups. Twenty min before stress, five of the groups received naltrexone (5 mg/kg, s.c.), while the remaining animals received saline control injections. Immediately before stress, all animals were tested for baseline pain responsiveness using the tail-flick test. The naltrexone-treated and control animals were then exposed to 3 min of continuous footshock (60 Hz sine waves) at either 1.5, 2.0, 2.5, 3.0 and 3.5 mA. Beginning 1 min after footshock cessation, all animals were tail-flick tested at 1 min intervals for 10 min. Data were analyzed using separate two-way analyses of variance for each of the five current intensities.

Naltrexone significantly ($p<.05$) reduced stress analgesia at 1.5 and 2.0 mA without attenuating stress analgesia elicited by 2.5, 3.0, or 3.5 mA.

These data show the importance of current intensity in differentially producing opioid and nonopioid stress analgesia. Viewed in the light of our previous findings concerning a similar role for footshock duration, it seems that the interaction between duration and intensity determines which neurochemically discrete substrate of stress analgesia is activated. (Supported by NIH grant NS 07628 and a gift from the Brotman Foundation)

232.23 THE EFFECTS OF DECEREBRATION AND SPINAL TRANSECTION ON THREE DISCRETE FORMS OF STRESS-INDUCED ANALGESIA. M. V. Klein*, K.M. Lovaas*, G.W. Terman and J.C. Liebeskind. Dept. of Psychology, UCLA, Los Angeles, CA 90024.

Numerous stressors have been reported to reduce responsiveness to noxious stimuli. We have recently reported that three distinct forms of such stress-induced analgesia can be differentially produced by varying the temporal parameters of a 2.5 mA inescapable footshock. Specifically, 20 min of intermittent footshock produces an analgesia that appears to be mediated by opioid peptides and is dependent on the integrity of the adrenal gland. One min of continuous footshock, on the other hand, elicits an opioid-mediated analgesia that is independent of the pituitary-adrenal axis. Four min of continuous footshock produces a third, nonopioid, form of stress analgesia. In this series of experiments, we attempted to define the anatomical pathways underlying these three neurochemically distinct analgetic phenomena.

In Experiment 1, 24 male Sprague-Dawley rats were assigned to two equal groups and injected with pentobarbital (55 mg/kg, i.p.). One group was spinally transected (T1) whereas the remaining animals were sham operated and served as controls.

In Experiment 2, 30 male Sprague-Dawley rats were divided into two equal groups and either decerebrated at the mid-collicular level or sham operated, under pentobarbital anesthesia.

On three consecutive days starting two days after surgery, animals in both experiments were exposed in a counterbalanced fashion to the three footshock parameters described above. Poststress analgesia was measured at 1 min intervals for 10 min using the tail-flick test. Data for each experiment were analyzed using separate two-way analyses of variance for each stress paradigm.

In Experiment 1, spinal transection significantly ($p<.05$) reduced all three forms of stress analgesia, suggesting a role for descending controls from the brain in these phenomena. In Experiment 2, decerebration significantly attenuated only the adrenal-dependent opioid form of stress analgesia, indicating the independence of the other two forms of stress analgesia from control by supra-mesencephalic brain structures. (Supported by NIH grant NS07628 and a gift from the Brotman Foundation).

232.24 CONDITIONED TOLERANCE TO ANALGESIA PRODUCED BY ELECTRICAL STIMULATION OF THE PERIACQUEDUCTAL GRAY IN RATS. D.J. Paul* and A.G. Phillips. Dept. of Psychology, Univ. of British Columbia, Vancouver, Canada, V6T 1W5.

Stimulation of the periacqueductal gray (PAG) of the midbrain produces an analgesia that resembles opiate analgesia. There is considerable behavioral and physiological evidence to suggest a commonality in the underlying mechanism for stimulation produced analgesia (SPA) and opiate analgesia. Tolerance to the analgesic effect of opiate drugs has been shown to be subject to the laws of classical conditioning. Specifically, certain aspects of opiate tolerance have been related to a compensatory physiological response elicited by cues that reliably predict the drug effect.

Given the apparent similarity between SPA and opiate analgesia, the present experiments test the hypothesis that tolerance to SPA also should be subject to the laws of classical conditioning. Electrical stimulation of the PAG increased tail-flick latencies from a baseline score of 2.9 sec to 7.5 sec. Brain-stimulation was delivered for 5 min per day in the presence of distinctive environmental cues and tolerance to SPA developed over 7 daily sessions (tail-flick latency on day 7, $\bar{x} = 3.7$ sec.) Half of the rats (N=8) were then given 12 extinction trials which consisted of exposure to the environmental cues, without brain-stimulation. The remaining subjects stayed in their home cages throughout the period. When tested again with brain-stimulation in the experimental environment, animals in the extinction group displayed significant SPA (i.e. tail-flick latency, $\bar{x} = 5.8$). The home cage group remained tolerant with a mean tail-flick latency of 3.2 sec. Reaquisition of tolerance was observed in the extinction group.

A second experiment examined the specific hypothesis that tolerance to SPA should be accompanied by the development of a hyperalgesic response. Decreasing the temperature of the water bath from 52°C to 48.5°C elevated baseline tail-flick latencies to $\bar{x} = 8.2$ sec. PAG stimulation produced strong analgesia with complete tolerance developing over 7 daily sessions. On the eight session half of the animals (N=8) were tested without brain-stimulation and a hyperalgesic response was observed (i.e. tail-flick latency, $\bar{x} = 5.8$ sec., $p < 0.05$).

These results provide further confirmation of the conditioning theory of tolerance and suggest that opiate analgesia and SPA share a common mechanism.

233.1 REDUCTION OF HIBERNATION BOUT DURATION BY ICV INFUSION OF NALOXONE IN CITELLUS LATERALIS. Carmen Llados-Eckman* and Alexander L. Beckman. Alfred I. duPont Institute, Wilmington, DE 19899.

In previous work (Science 212:1527,1981), we demonstrated that opioid (morphine) physical dependence does not occur during deep hibernation. Others have shown that the CNS endogenous opioid system may be involved in the control of behavioral states and energy balance, two variables that are profoundly altered during hibernation. These findings raise the question as to whether the endogenous opioid system participates in controlling the state of hibernation. To explore this possibility, we attempted to interfere with the endogenous opioid system during hibernation by the constant intracerebroventricular (ICV) infusion of naloxone.

Hibernating ground squirrels (C. lateralis) of either sex were infused with naloxone hydrochloride, starting on the first day of the hibernation bout and lasting throughout the bout, by means of an osmotic minipump (Alza Corp.). The pump, immersed in a 0.9% NaCl bath, delivered naloxone through a length of PE 20 polyethylene tubing to a previously implanted cannula guide tube (21 ga.; 26 ga. injection tube) into the lateral cerebral ventricle. Doses infused were 1 µg/µl, 5 µg/µl, 7.5 µg/µl or 10 µg/µl at a flow rate of 1 µl/hr. Control animals were similarly infused with 0.9% NaCl. The length of each bout and the behavioral changes in the period immediately following arousal from hibernation were recorded.

In all animals, naloxone shortened the duration of the hibernation bout in relation to the immediately previous bout in a dose-dependent manner. Expressed as the percentage (± SEM) of bout shortening, the data were: 6.5 ± 4.4% (0.9% NaCl, control), 13.9 ± 4.2% (naloxone, 1 µg/hr), 47.7 ± 1.9% (naloxone, 5 µg/hr), and 62.3 ± 2.4% (naloxone, 7.5 µg/hr). The highest dose of naloxone (10 µg/hr), tested in only two animals, shortened the bout by an average of 47% and produced convulsions and abstinence-like signs in the arousal and post-arousal periods. One of these two animals died following arousal.

These data suggest that component neurons of the endogenous opioid system contribute to the maintenance of the hibernation state in C. lateralis. (Supported by NIDA grant DA-02254 and the A.I. duPont Institute).

233.2 TAIL SKIN TEMPERATURE: A SENSITIVE IN VIVO BIOASSAY FOR OPIATE RECEPTOR ANTAGONISTS. J.W. Simpkins*, I.C. Song*, M.J. Katovich*, N. Bodor* and R. Tuttle* (Spon: W.Millard) College of Pharmacy, University of Florida, Gainesville, FL 32610.

We have recently observed that in morphine-dependent rats, naloxone administration causes a prompt, dramatic elevation in tail skin temperature (Simpkins et al., Life Sciences 32: 1957, 1983). In the present study we evaluated this tail skin temperature (TST) response as a potential in vivo bioassay for narcotic antagonists. Morphine (MOR) dependency was produced by s.c. implantation of a single pellet containing 75 mg of MOR (freebase) followed 2 days later by 2 additional pellets. After a total of 4 days of exposure to MOR, animals were lightly restrained in wire-mesh cages and thermisters were attached to the dorsal surface of the tail. After a 30 min acclimation period, TST was recorded at 10 min intervals for 30 min to establish basal TST. Animals then received 1 of 5 doses of naloxone (NAL; 0, 0.01, 0.1, 0.5 or 1.0 mg/kg body weight, s.c.), naltrexone (NALT; 0, 0.001, 0.005, 0.01, 0.02, 0.1 mg/kg body weight, s.c.), a 6-spiro thiazolidine derivative of naltrexone (NALT-CYS; 0.001, 0.005, 0.01, 0.02 or 0.1 mg/kg body weight, s.c.) or nalmefene (JF-1; 0.001, 0.005, 0.01, 0.02, or 0.1 mg/kg body weight, s.c.). TST's were recorded at 5 min intervals for an additional 90 min following administration of the narcotic antagonist. Six different drug naive rats were used at each dose level for each of 4 narcotic antagonists studied.

Each of the narcotic antagonists caused a dose-dependent increase in TST. Peak TST were observed at 15 to 20 min and TST returned to the pre-injection baseline by 60 to 90 min after administration of the narcotic antagonists. For each drug evaluated, a linear relationship between the dose and the maximum change (Δ MAX) in TST was observed. A similar linear relationship was observed when drug dose and the area under the TST response curve was evaluated. The dose required to elevate TST to 50% of maximum (ED50) was 115 µg/kg for NAL, 30 µg/kg for NALT-CYS, 18 µg/kg for NALT and 8 µg/kg for JF-1. The relative estimate of potency of these narcotic antagonists obtained in this study is similar to that determined by other bioassay methods. In view of the ease of quantitation and the high sensitivity of the TST response to narcotic antagonists, this method could be readily applied to the evaluation of compounds with potential narcotic antagonist activity.

233.3 THE EFFECTS OF IN UTERO EXPOSURE TO MORPHINE ON POSTNATAL THYROID AXIS FUNCTION IN THE RAT. W.J. Litto* and J. Rabii. Dept. Biol. Sci. (Physiol.) and Bureau of Biol. Research, Rutgers Univ., Piscataway, NJ 08854.

Recent studies in our laboratory have indicated that opiates, in addition to their established acute actions, can produce long-lasting alterations in the development of neuroendocrine control of the pituitary. Results presented here are a continuation of these studies. We have examined the effects of injecting pregnant rats with morphine sulfate (MS) on days 5-12 of gestation, on the thyroid axis response of the offspring to changes in environmental temperature. Groups of MS- or vehicle-treated rats from 10 to 35 days of age were maintained at 22°C or exposed to 4°C or 37.5°C for 45 and 60 minute periods, respectively. At the end of each treatment trunk blood was collected and assayed for thyrotropin (TSH) and thyroxine (T_4) by RIA. In 10-day-old male and female pups both 4°C and 37.5°C temperature treatments inhibited TSH secretion as compared to 22°C controls, in prenatal saline treated animals. The opposite trend was observed in 10-day-old MS-treated pups, where 4°C and 37.5°C resulted in enhanced TSH secretion compared to room temperature. Exposure to 37.5°C produced an inhibition of TSH secretion, in male and female pups of both prenatal treatment groups, from 15 to 35 days of age. A more pronounced inhibition of TSH was observed in MS-treated male and female pups at 15, 20, and 35 days of age. Exposure to 4°C resulted in an enhancement of TSH secretion for pups of both sexes and both prenatal treatment groups from 15 to 35 days. Prenatal MS male and female pups showed a greater TSH response to cold at 15 and 25 days than their corresponding prenatal vehicle-treated groups, and a marked attenuation of this response at 20 and 35 days of age. Exposure to 37.5°C resulted in a marked increase in T_4 plasma levels in 10-day-old saline treated pups of both sexes. The opposite result occurred in the MS-treated animals where significantly lower T_4 levels were observed in heat-exposed pups of both sexes. Cold exposure had no significant effect on T_4 levels at 10 days in any of the prenatal treatment groups. Elevated T_4 levels were noted in cold-exposed 35-day-old saline-treated pups of both sexes. This pattern was not observed in 35-day-old male or female morphine treated pups. In conclusion, these results indicate that in utero exposure to MS can produce alterations in the thyroid axis response to changes in environmental temperature after birth.
Supported by NIH grant DA02227 and the Rutgers University Bureau of Biological Research.

233.4 LACK OF CORRELATION BETWEEN LUTROPIN (LH) AND FOLLITROPIN (FSH) SECRETORY PATTERNS UNDER PROLONGED BLOCKADE OF ENDOGENOUS OPIOID SYSTEMS IN NORMAL MEN. J. Ellingboe, J.D. Veldhuis*§, J.H. Mendelson, J.C. Kuehnle* and B. Andrews*. Alcohol & Drug Abuse Research Center, McLean Hospital-Harvard Medical School, Belmont, MA 02178; and §Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville, VA 22908.

Blockade of endogenous opioid activity by naltrexone (NTX) has been shown to increase pulsatile LH secretion, but little is known about concomitant FSH release, which is believed to be controlled by the same hypothalamic factor, luliberin (LHRH).

Plasma gonadotropin levels were assessed on the day after placebo (P) and NTX administration in 2 groups (G1 and G2). G1 received P at 1800 h on Study Day 2, and 20-min serial integrated samples of blood were taken via intravenous catheter from 0900 h to 1520 h on Day 3. NTX (50 mg oral dose) was given at 1800 h on Day 4, and serial blood samples were collected again on Day 5. The order of P/NTX administration was reversed for G2. Plasma LH and FSH results are presented as ng LER-907 standard per ml ± SEM. FSH concentrations are shown in brackets.

Plasma LH after NTX was higher than after P in 14 of the 17 subjects for whom LH was analyzed, while FSH was higher after NTX in 16 of 21 subjects. G1 included 3 of the subjects with decreased LH after NTX and 4 subjects with no change or decreased FSH after NTX; but only one subject had decreases in both LH and FSH (ca.-4%). After P, LH [FSH] averaged 48±4 [549±53] for G1 and 43 ±3 [577±51] for G2. After NTX, LH [FSH] levels were 50±3 [572 ± 58] for G1, and 65±6 [565±55] for G2. Only in G2 did LH and FSH levels rise significantly above control levels after NTX treatment. LH was significantly higher in G2 than in G1 after NTX, but no significant differences in LH were found in G1 versus G2 after P, nor were there differences in FSH between G1 and G2 after NTX or P. The mean percent rise in LH from nadir to peak was greater after P than after NTX in G1 (87% versus 65%, p 0.05). Over the period of blood collection there were averages of 3.0 LH pulses and 2.0 FSH pulses, whether after P or NTX, or in G1 and G2. There was little coincidence between LH and FSH pulses; only one in six LH pulses occurred simultaneously with an FSH pulse, whether after P or after NTX. Interestingly, 3 days after NTX, LH pulses became markedly synchronized among subjects in G2, with a peak at 1230 h and nadirs at 0950 h and 1420 h.

Although the data indicate that endogenous opioids inhibit LHRH secretion, as suggested by prolonged increases of both LH and FSH after NTX, other factors must alter LH and FSH secretion differentially, perhaps at the pituitary level, with or without opioid blockade. Differences between the two experimental groups may reflect individual variation, or perhaps a sequence effect.
Supported by Grant DA-01676 from NIDA.

233.5 MEPTAZINOL: A UNIQUE MU_1 SELECTIVE OPIATE. K. Spiegel and G.W. Pasternak. Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021, USA.

Meptazinol, WY22811, is an unusual analgesic agent. Although its analgesic actions can be reversed with the antagonist naloxone, it displaces ^{3}H-opioid binding poorly. In an effort to address this problem, we have examined the binding, analgesic and respiratory depressant actions of meptazinol. As previously noted, meptazinol was not a potent inhibitor of ^{3}H-labeled opioid binding with IC_{50} values well over 50 nM for dihydromorphine, ethylketocyclazocine and SKF10047 and greater than 300 nM for D-ala^2-D-leu^5-enkephalin. However, detailed displacements were multiphasic, with meptazinol at concentrations under 10 nM inhibiting a portion of the binding for all ^{3}H-ligands. Blockade of mu_1 sites in vitro with naloxonazine eliminated this initial displacement. This selectivity of meptazinol for mu_1 sites was supported by the ability of meptazinol at 5 nM to selectively inhibit the high affinity, or mu_1 binding of ^{3}H-dihydromorphine in saturation experiments. Blockade of mu_1 sites in vivo with naloxonazine significantly attenuated meptazinol's analgesic actions. Naloxonazine shifted the dose-response curve for meptazinol in the writhing assay 2.6-fold (4.6 to 12 mg/kg). Similarly, the analgesic action of 10 mg/kg (i.v.) of meptazinol in the rat, which were equivalent to 3.5 mg/kg of morphine sulfate, were markedly attenuated by naloxonazine treatment. Previous work from our laboratory has suggested that spinal mechanisms for opioid analgesia in the mouse do not involv mu_1 sites. As expected, spinal transection eliminated meptazinol analgesia. Whereas the ED_{50} value in control mice was 44 mg/kg in the tail-flick assay, no analgesia was seen with doses of meptazinol of 40 and 60 mg/kg in spinalized mice. Mu_1 sites also appear not to mediate morphine's respiratory depression. Thus, it was not surprising that at an equianalgesic doses, meptazinol showed no respiratory depression whereas morphine significantly depressed pO_2 and pH and elevated pCO_2, as measured from arterial blood samples. These results suggest that meptazinol's pharmacological actions might result from selective interactions with mu_1 sites.

233.6 DEPRESSION OF ADENYLATE CYCLASE IN SYMPATHETIC PREGANGLIONIC NEURONS BY CLONIDINE AND BY OPIATES. Donald N. Franz, Parley W. Madsen, and Bradford D. Hare*. Departments of Pharmacology and Anesthesiology, University of Utah, Salt Lake City, Utah 84132.

The suppression of opiate-withdrawal symptoms by clonidine has been attributed to common effects of clonidine and opiates on central neurons having both alpha-2 adrenergic and opiate receptors. We have found that sympathetic preganglionic neurons (SPGNs) are depressed by both clonidine and opiates acting on their respective receptors and have suggested that these neurons are important sites for the antiwithdrawal effect of clonidine (Science 215:1643, 1982). Since clonidine appears to depress SPGNs by inhibiting adenylate cyclase and reducing cyclic AMP levels (Neurosci. Lett. 28:211, 1982; Soc. Neurosci. Abstr. 8:426, 1982), the possibility that opiates also depress SPGNs by inhibiting adenylate cyclase was investigated.

Sympathetic discharges recorded from upper thoracic preganglionic rami were evoked at 0.1 Hz by stimulation of descending excitatory pathways in the cervical dorsolateral funiculus of unanesthetized, spinal cats. Inhibition of cyclic AMP phosphodiesterase (PDE) by aminophylline (50 mg/kg), IBMX (1 mg/kg), or RO 20-1724 (1 mg/kg) enhanced intraspinal transmission to about 175% of control values within 10 min. Pretreatment with clonidine HCl (25 ug/kg) rapidly depressed transmission to about 20% of control and completely prevented enhancement by the PDE inhibitors. The alpha-2 receptor antagonists, yohimbine (0.5 mg/kg) or tolazoline (2.5 mg/kg), rapidly reversed the depression by clonidine and restored the ability of the PDE inhibitors to enhance transmission. Morphine (2 mg/kg) or methadone (1 mg/kg) also depressed transmission to 0-40% of control and almost completely suppressed enhancement by the PDE inhibitors. The opiate antagonists, naloxone or naltrexone (20-40 ug/kg), rapidly reversed the depression by the opiates and restored the ability of the PDE inhibitors to enhance transmission. These results support the proposal that clonidine and opiates depress SPGNs by activating alpha-2 and opiate receptors, both of which are negatively coupled to adenylate cyclase. Clonidine may suppress opiate withdrawal by substituting for opiates as an inhibitor of adenylate cyclase in SPGNs and in other central neurons.

(Supported by NIH grants HL-24085, RR-05428, and GM-07579)

233.7 CHANGES IN CORTICAL ADRENOCEPTOR FUNCTION FOLLOWING CHRONIC MORPHINE ADMINISTRATION IN RAT. H.C. Moises and C.B. Smith.*+ Dept. of Physiology and Dept. of Pharmacology,+ Univ. of Michigan, Ann Arbor, MI 48109.

Administration of opiates interferes with noradrenergic neurotransmission in the brain. In this study, radioligand binding techniques were used in conjunction with in vivo electrophysiological recording to determine whether changes occur in the density and/or sensitivity of central adrenergic receptors following long-term opiate treatment. The maximum number of specific binding sites (B_{max}) and dissociation constants (K_D) for ^{3}H-clonidine (CLON), an $alpha_2$ adrenoceptor agonist, and for ^{3}H-dihydroalprenolol (DHA), a beta receptor antagonist, were measured with neural membranes prepared from parietal cortex, hippocampus, and cerebellum of morphine- or saline-treated rats. Rats were injected with morphine or saline, i.p., every 8 hr for 14 days, with the dosage of the narcotic ranging from 10 mg/kg, t.i.d., on the first 3 days to 100 mg/kg, t.i.d., on the last 2 days. In control experiments, the B_{max}'s for specific binding of CLON and DHA, respectively, were (in fmoles/mg protein): parietal cortex, 130±4 and 71±5; hippocampus, 94±2 and 41±2; and cerebellum, 27±3 and 49±10. After chronic morphine treatment, the B_{max} for CLON was decreased significantly ($P<.05$) in parietal cortex (20%), but increased in cerebellum (27%, $p<.05$). Specific DHA binding showed a significant change ($p<.025$) only in parietal cortex, increasing by 24%. Morphine treatment did not alter the K_D's for either ligand.

Changes in sensitivity of postsynaptic adrenoceptors following chronic morphine were assessed by comparing the mean threshold current of iontophoretic norepinephrine (NE) (10 sec pulses, 250 mM conc.) required to inhibit the firing of cerebrocortical neurons recorded in drug-treated and saline control rats. In individual experiments, the same 5-barreled micropipet (3-8 MΩ) was used to alternately record single units from tandemly prepared, anesthetized control and morphine-treated animals. In tests on 26 cell pairs from 12 experiments, the mean threshold NE current required for depression of neurons in morphine-treated animals (20.6±3.8nA, S.D.) was significantly less ($p<.01$) than that of control cells (34.2±6.1nA, S.D.). Neurons recorded from morphine-treated rats displayed a similar increase in sensitivity to the inhibitory effects of isoproterenol (N=12 pairs). In comparison, no significant change in neuronal responsiveness to gamma aminobutyric acid was observed after chronic morphine treatment. Taken together, these findings suggest that changes in adrenoceptors in specific brain regions may be important in the development of narcotic

233.8 OPIATE INHIBITION OF CCK-8 RELEASE FROM HYPOTHALAMUS AND CORTEX OF ZUCKER LEAN AND OBESE RATS, IN VITRO. P. Micevych, T. Yaksh and V. L. W. Go*. Depts. of Neurosurgery and Gastroenterology, Mayo Foundation, Rochester, MN 55905.

Recent evidence has implicated both the endogenous opioid peptides and cholecystokinin octapeptide (CCK-8) in the regulation of feeding behavior. Opiates inhibit the stimulated release of CCK-8 from hypothalamus, in vitro, thus the opiate effects on feeding behavior may be modulated through a regulation of CCK-8 release in the hypothalamus. We speculated that obese Zucker rats (fa/-) which have an exaggerated stimulated release of CCK-8 may have an altered opiate modulation. Age-matched lean (200-270g) and obese (450-550g) Zucker rat littermates were decapitated and the hypothalamus and frontal cortex dissected out. Hypothalamic or cortical fragments were superfused with oxygenated KRB containing D-glucose and bacitracin. Samples were analyzed by RIA. Drug-free levels of release from lean rat hypothalamus were 1.15 ± 0.16 fmole CCK-8 equivalents/mg/10 min and from the obese rat 2.1 ± 0.19 fmole CCK-8 equivalents/mg/10 min. The percent inhibitions of stimulated (50mM K^+) CCK-8 release from lean rat hypothalamus with morphine (10nM) was 52% and with d-ala^2-d-leu^5-enkephalin (DADLE, 10nM) was 53%. The percent inhibition of stimulated CCK-8 release from obese rat hypothalamus was 77% with morphine (10nM) and 66% with DADLE (10nM). The cortical release of CCK-8 was 2.6 ± 0.35 from lean rats and 2.7 ± 0.41 fmole CCK-8 equivalents/mg/10 min from obese rats. Morphine and DADLE did not inhibit the stimulated cortical release of CCK-8. Equimolar concentrations of the specific opiate antagonist, naloxone, blocked the opiate inhibition of both morphine and DADLE in the lean and obese rats. Sephadex G-50 chromatography demonstrated that the immunoreactive CCK had a similar retention co-efficient to authentic sulfated CCK-8. These results indicate that the CCK-containing neurons of the hypothalamus of both lean and obese rats are sensitive to opiate inhibition of release while cortical CCK neurons are not. However, obese rats may have an altered degree of sensitivity to opiates, with respect to the release of CCK-8 which was not revealed in these experiments. The exaggerated release of CCK-8 from obese rat hypothalamus, in vitro thus may involve an altered "fine-tuning" within the hypothalamus.

(Supported by NS16541 (T.Y.), AM07198-06 (V.G.) and the Mayo Foundation).

233.9 DO OPIOIDS MODULATE NEURALLY MEDIATED ADRENAL OR PLASMA CATECHOLAMINE RELEASE? Karen L. Cochrane,* K. Bridget Brosnihan, Ph.D. and Carlos M. Ferrario, M.D. Cleveland State University and Research Division, Cleveland Clinic Foundation, Cleveland, Ohio 44106.

To delineate whether opioids modulate the neural release of adrenal catecholamines, we have stimulated the preganglionic splanchnic nerve before and after systemic administration of either an opiate agonist or an antagonist. Sixteen dogs were anesthetized with pentobarbital (30 mg/kg, IV) anesthesia. Adrenal secretion rate was assessed in situ. The preganglionic splanchnic nerve was stimulated at a fixed voltage and pulse duration (10V, 1 msec duration), and variable frequencies ranging from 0.3 to 10.0 Hz before and after administration of morphine (2 mg/kg IM) or naloxone (2 mg IV). Epinephrine and norepinephrine were measured by a radioenzymatic assay. Basal epinephrine secretion rates (22.7 ± 11.0 ng/min) and norepinephrine secretion rate (2.9 ± 1.1 ng/min) were unaffected by the administration of either drug. On the other hand, the dose stimulus response curve relating secretion rates of epinephrine and norepinephrine as a function of stimulus frequency was shifted to the left of the control curve by naloxone ($F = 7.52$, $p < 0.05$ and $F = 7.11$, $p < 0.05$, respectively). Morphine treatment was without effect on the neurally mediated release of adrenal catecholamines. We also observed that treatment with morphine reduced circulating plasma norepinephrine levels in both basal conditions and at the threshold frequency of stimulation, but markedly elevated plasma norepinephrine levels at higher frequencies of stimulation. These studies provide evidence for a role of endogenous opioid peptides tonically inhibiting the neurally mediated release of adrenal catecholamines in dogs anesthetized with pentobarbital. In addition, the potentiating effect of morphine on plasma norepinephrine level was unexpected but may be consistent with opioids acting at sites innervated by the splanchnic nerve other than the adrenal medulla although more indirect modes of action cannot be eliminated. (Supported in part by NHLBI grants HL-6835 and HL-24100).

233.10 CHRONIC OPIATE ADMINISTRATION INCREASES THE BINDING OF ^{3}H-CLONIDINE TO ALPHA-2-ADRENERGIC RECEPTORS IN CULTURED MURINE BRAIN AND CEREBRAL CORTEX. R. J. Plishka and J. H. Neale. The Department of Biology, Georgetown University, Washington, D. C. 20057.

Clonidine, a potent alpha-2-adrenergic receptor agonist, ameliorates the symptoms of opiate withdrawal. The mechanisms proposed for this action have focused upon the adrenergic neurons of the locus coeruleus, which possesses both opiate and alpha-2-adrenergic receptors. Chronic morphine administration is reported to increase alpha-2-adrenergic receptors in rat cerebral cortex in vivo.

We report here that chronic morphine administration to brain cell cultures (75 uM morphine sulfate; day 24 through day 30 in culture) caused a 24% increase in the binding of ^{3}H-clonidine. Cells from cerebral cortex which were grown in culture without benefit of adrenergic input also demonstrated a substantial increase in alpha-2-adrenergic receptor binding as a result of chronic morphine administration. In contrast, neither cell cultures prepared from brain without cortex nor the neuroblastoma-glioma hybrid cell line, NG108-15, which also possesses both opiate and alpha-2-adrenergic receptors, responded to chronic morphine administration with significant changes in ^{3}H-clonidine binding.

233.11 AN IN VIVO ELECTROCHEMICAL AND BEHAVIORAL STUDY OF THE EFFECT OF D-ALA^2-D-PRO^5-ENKEPHALINAMIDE ON DOPAMINE RELEASE FROM RAT TUBERCULUM OLFACTORIUM. F.A. Broderick, E.L. Gardner and H.M. van Praag*. Depts. of Psychiatry and Neurosci., Albert Einstein College of Medicine, Bronx, NY 10461

Rat tuberculum olfactorium is a mesolimbic brain area which has been shown to mediate, at least in part, the hyperactivity induced by amphetamine (Pijnenberg, et al, Eur. J. Pharm. 35:1976). Recent behavioral evidence has shown an enkephalinergic modulation of dopaminergic function in mediating mesolimbic components of amphetamine-induced locomotor activity (Broderick et al, Soc. Neurosci. Abstr. 8:1982). The purpose of the present study was to examine the effect of an enkephalin pentapeptide analog, D-Ala^2-D-Pro^5-enkephalinamide (DAP) (Wy 42,186), on the locomotor activity induced by d-amphetamine sulfate (AMP) and on dopamine release from the tuberculum olfactorium as measured by in vivo electrochemistry.

Male, Sprague-Dawley rats (350-450 g), group housed, fed and watered ad lib, were treated with DAP (5 mg/kg i.p.), followed one-half hour later with AMP (2.5 mg/kg i.p.). Testing for locomotor activity, as shown by the number of crossings into separate quadrants of the testing apparatus, began 15 min after AMP injection and continued for 2 min sessions every 20 min for 2 hours. The rats were isolated in a sound proof room and were observed through an adjoining one-way mirror. Semiderivative voltammograms were recorded every ten min at a scan rate of 10 mv/sec. Catecholamine sensitive electrodes (Blaha and Lane, Br. Res. Bull., in press, 1983) were stereotaxically implanted in the tuberculum olfactorium of chloral hydrate anesthetized rats, controlled for temperature changes. A Ag/AgCl reference electrode and a Pt auxiliary electrode were placed in contact with the cortex. After a stable, steady state dopamine signal was achieved, DAP (5 mg/kg i.p.) was administered. The effect of DAP on the dopamine signal was investigated for a period of one to two hours.

The results showed that DAP produced no significant alterations on dopamine release in rat tuberculum olfactorium, and produced no significant change in amphetamine-induced locomotor activity. Moreover, DAP was shown to significantly inhibit dopamine release from rat striatum and to have no effect on dopamine release from rat nucleus accumbens (Broderick et al, Biol. Psychiat., in press, 1983). These data further show a site specific enkephalinergic modulation of dopamine both behaviorally and biochemically. These results also suggest a correlation between mesolimbic dopaminergic function and locomotor activity induced by amphetamine. (Supported by USPHS Grant MH 15788).

233.12 CRF-INDUCED ACTH/β-END RELEASE IN STRESS. E.A. Young,* S. Watson, and H. Akil (SPON: G.C. Quarton). Mental Health Res. Inst., Univ. of Michigan, Ann Arbor, MI 48109.

The activation of the hypothalamo-pituitary-adrenal (HPA) axis in stress is a classic endocrinological finding. The adrenal steroids produced by this activation inhibit the further release of ACTH from the pituitary (feedback inhibition). Glucocortioids appear to be active at both the hypothalamus and pituitary to suppress release of both corticotropin releasing factor (CRF) and ACTH. The demonstration that an opioid peptide, β-endorphin (β-END) and ACTH are cosynthesized and co-released in pituitary suggests that β-END may also be released during stress.

Acute intermitent footshock has been demonstrated to produce analgesia in rats that is naloxone reversible. When this same shock is continued for 14 days, (chronic stress) the animals become tolerant to the analgesic effects of stress. Analgesia produced by footshock is attenuated by hypophysectomy and adrenalectomy, again suggesting the involvement of the HPA axis.

Since intermittent footshock has been well characterized, we were interested in studying the effects of these acute and chronic stress paradigms on the ability of the pituitary to respond to CRF and arginine vasopressin (another ACTH releaser). Pituitaries were removed from rats who had received either acute stress, chronic stress with the last stress occuring 24 hours prior to decapitation, and chronic stress followed by an acute stress immediately prior to decapitation, (chronic stress/acute stress). Pituitaries from naive, unstressed animals were used as the control group. After processing into single cell preparations, the pituitaries were incubated with varying doses of CRF (10^{-11} M to 10^{-9} M) and vasopressin (10^{-10} M to 10^{-8} M). Release of ACTH and β-END into the medium was measured by RIA. A clear response to both releasers was seen in control pituitary. In acute stress, a decreased responsivity to vasopressin and CRF is seen. This same blunted release is not seen in chronic stress, even if the animals are stressed immediately prior to decapitation. At the higher doses of CRF (10^{-9}M) an increased release of ACTH and β-END is seen in the chronically stressed rats. Thus it would appear that there is a loss of the normal steroid feedback inhibition on pituitary release of ACTH. In addition there may be changes in the ability of the pituitary to readily release ACTH (releasable pools) in chronic stress.

This work was supported by NIDA grant DA02265 and NIMH grant MH 36168 to HA and SJW.

233.13 CHANGES IN β-ENDORPHIN CONCENTRATIONS IN THE MEDIAL PREOPTIC AREA DURING PREGNANCY IN THE RAT. R.S. Bridges and P.M. Ronsheim*. Department of Anatomy, Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School, Boston, MA 02115.

The expression of maternal behavior in the rat can be disrupted by the administration of the opiate morphine. Recently, we have reported that direct application of morphine to the medial preoptic area (mPOA), an area known to regulate maternal behavior in rats, interferes with ongoing maternal behavior. Based upon these findings and the reports of increased pain thresholds (Science 210:193, 1980) and elevated concentrations of β-endorphin (βE) in whole hypothalami (Brain Research 245:327, 1982) in pregnant rats, we have proposed that the onset of maternal behavior at parturition may result from a decline in endogenous opioid activity prepartum. In the present study we have addressed this possibility by measuring the content of the endogenous opiate, βE, during pregnancy in a region of the CNS identified with the control of maternal behavior, the mPOA, and in the medial basal hypothalamus (MBH). Tissue samples from the mPOA and MBH were obtained by macrodissection from pregnant rats (N = 6-7/group) between 1000 and 1400 h on days 6, 12, 18 and 22 of gestation. Samples were also collected from nonpregnant females on the morning of diestrus. Tissues were homogenized on ice in 0.2% HCl/1% formic acid, and the homogenates stored frozen at -20°C. Immediately prior to assay determinations of βE concentrations, each sample was thawed and an aliquot of the homogenate taken for protein determination (Bradford method). The remaining sample was microfuged at 8800 g and aliquots of the supernatant taken for βE measurement by radioimmunoassay. βE levels in the mPOA of 6, 12 and 18 day pregnant rats were higher than those found in diestrous rats. Concentrations of βE rose from a mean of 8.5 ng βE/mg protein on diestrus to 14.3 ng/mg on day 6 of gestation. Levels of βE remained elevated on days 12 and 18 of pregnancy; the average concentration of βE for days 6, 12 and 18 was 14.2 ± 1.3 ng/mg. βE content in the mPOA was significantly lower on day 22 ($\bar{X}$ ± SE = 9.0 ± 2.2 ng/mg) than during the other times of pregnancy. In contrast to the changes in βE in the mPOA found over the course of pregnancy, significant changes in βE concentrations were not detected in the MBH either over the course of pregnancy or as a function of the state of pregnancy. Mean βE levels in the MBH of pregnant and diestrous rats were approximately 22-24 ng/mg. Although the functional relationship of opioid concentrations to its activity is unproven, these findings are consonant with those indicating that a reduction in opioid concentrations/activity in the mPOA prepartum stimulates maternal behavior at parturition. (Supported by BOC Grant #5-311 from the March of Dimes Birth Defects Foundation and by NSF Grant BNS 80-14670 awarded to RSB.)

233.14 EFFECT OF ESTROGEN AND PROGESTERONE ON THE RAT BRAIN AND PITUITARY OPIOIDS. G.A. Tejwani, K.K. Vaswani,* J.C. Barbacci,* C.W. Richard III, and J.R. Bianchine. Dept. of Pharmacology, The Ohio State Univ. College of Medicine, Columbus, Ohio 43210

Morphine and endogenous opioids have been shown to decrease the release of gonadotropins in various species including rat. The opiate antagonist, naloxone, facilitates the release of pituitary gonadotropins. This effect of naloxone is blocked by estrogens suggesting that gonadal steroids influence the secretion of gonadotropins by altering the level of endogenous opioids. This prompted us to investigate the effect of progesterone in presence and absence of estradiol on the level of β-endorphin in rat pituitary, hypothalamus and striatum. Forty-eight female Sprague Dawley rats were divided in 2 equal groups for acute and chronic studies. Each group was further divided into 4 subgroups, each containing 6 animals. Each rat in the first control group received an inert pill (15 µg in 0.25 ml corn oil daily by gavage); in second group norethindrone (NE, progesterone, 15 µg MICRONOR); in third group 15 µg NE and 1 µg ethinyl estradiol (EE_2 MEDICON); in fourth group 10 times the dose of the third group was given. Rats were treated either acutely for 5 days or chronically for 7 weeks. β-endorphin was estimated by a sensitive and specific RIA procedure developed in this laboratory. The concentration of β-endorphin-like immunoreactivity (β-EI) in pituitary, hypothalamus and striatum of control rats was 459±31, 10±2 and 0.98±0.07 ng/mg protein, respectively. In acute study, NE alone did not change β-EI significantly in pituitary. NE and EE_2 together decreased β-EI by 37% (47% at 10x dose). In chronic study, NE had no significant effect on pituitary β-EI. However, NE and EE_2 together increased β-EI by 15%, but at 10x dose decreased it by 14%. In the hypothalamus, NE alone or in presence of EE_2 had no significant effect on β-EI but 10x dose of NE+EE_2 caused 50 and 76% decrease in β-EI in acute and chronic study. Striatum was the only tissue where NE alone caused a decrease of 82% in β-EI when given acutely and 52% when given chronically. EE_2 had some protective effect on this decrease since when given together (NE+EE_2) the decrease in β-EI was 21% in acute and 43% in chronic study. Thus our results, along with other studies on the regulation of gonadotropin levels by opioids, suggest that oral contraceptives alter the level of β-EI and in turn may regulate the release of gonadotropins. (Supported by a grant from the Weight Watchers Fdn., Inc; and by the Roessler Fdn. of The Ohio State Univ.)

233.15 INCREASE OF STRIATAL MET^5ENKEPHALIN-ARG^6-PHE^7 (ME-Arg-Phe) CONTENT ELICITED BY LONGTERM TREATMENT WITH HALOPERIDOL. J. Chou*, J. Tang*, H.-Y.T. Yang and E. Costa (SPON: J. Stevens). Lab. Preclinical Pharmacology, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032

The elevation of Met^5-enkephalin (ME) content in striatum of rats receiving a daily injection of haloperidol for 3 weeks has been reported from this laboratory (Hong et al., JPET 205:141-147, 1978). Since Met^5-Enk-Arg^6-Phe^7 (ME-Arg-Phe) is derived from the same preproenkephalin A as ME, we have studied the effect of haloperidol on the ME-Arg-Phe content of various rat brain structure. We have studied in detail its striatal content, the release and accumulation rate after inhibition of ME-Arg-Phe degradation in rats receiving saline or haloperidol. ME-Arg-Phe content in striatum was increased (from 1.32+0.15 to 2.11+0.19 pmol/mg protein) in rats receiving haloperidol once daily for 2-3 weeks, and this increase was dose dependent. The latency time for the ME-Arg-Phe increase is similar to that of ME. Following intraventricular injection captopril, an inhibitor of ME-Arg-Phe degrading enzyme, the rate of striatal ME-Arg-Phe accumulation was greater in haloperidol (570 fmol/mg protein/hr) than in saline (200 fmol/mg protein/hr) treated rats. A slower rate of ME-Arg-Phe release in halopeidol treated rats can be excluded as a cause for this drug-induced increase in ME-Arg-Phe content because the peptide is released by a similar concentration of K^+ and at a similar rate in striatal slices prepared from haloperidol and saline treated rats. The parallel increase of ME-Arg-Phe and ME content after chronic treatment with haloperidol suggests that haloperidol increases the biosynthesis of the specific mRNA for preproenkephalin A, which causes an increase in the content of both ME-Arg-Phe and ME.

233.16 EFFECT OF POSTNATAL PHENOBARBITURATE TREATMENT ON ENKEPHALIN, METABOLISM BY RAT BRAIN ANIMO-AND ENDOPEPTIDASES. S. Mousa* S. Tjioe and D. Couri* Department of Pharmacology, College of Medicine, Ohio State University, Columbus, Ohio 43210.

It has been shown that in vitro barbiturates inhibited the degradation of enkephalin by rat striatum membrane-associated endopeptidase (enkephalinase A) (Life Sci., 28: 185, 1981). The effect of postnatal administration of phenobarbital to male and female Wistar rats in a dose of 45 mg/kg subcutaneously daily from day 1 postnatally (birth = day 0) (4 litters) thru day 19 on enkephalin degrading enzymes was examined. Brains from saline and phenobarbital treated rats were used to prepare aminopeptidases (high speed supernatant) and enkephalinase A (synaptosomal membrane preparation). Incubation of methionine-enkephalin (ME) with rat brain aminopeptidases liberated tyrosine (T) while incubating with enkephalinase A resulted in the formation of tyrosylglycylglycine (T-G-G). The separation and quantitation of T, T-G-G and ME was performed using a high performance liquid chromatography coupled to electrochemical detector (J. Chromatography, 1983, in press). The phenobarbital treatment resulted in a significant inhibition of enkephalinase A and a slight activation of aminopeptidases. Phenobarbital protects ME from degradation an effect which may be due to its inhibitory effect on enkephalinase A. Further study is in progress to test other barbiturates. A preliminary experiment with secobarbital showed a highly significant inhibitory effect on enkephalinase A and the subsequent protection of ME.

Treatment	Aminopeptidase Activity		Enkephalinase A Activity	
	Unit*	% ME Degraded	Unit*	% ME Degraded
Saline	60.9 ± 2.6	75.9 ± 1.2	68.6 ± 2.5	42.6 ± 7.5
Phenobarbital	83.3 ± 8.4	71.9 ± 3.4	22.1 ± 2.0	20.7 ± 3.3
	p < 0.025	NS	p < 0.001	p < 0.06

* unit = ug T or T-G-G formed/mg protein/hr

Supported by United Cerebral Palsy. Grant No. R-315-80.

234.1 INTERACTIONS OF DOPAMINERGIC NEURONS IN RAT RETINA WITH LIGHT: EFFECTS OF STIMULATION AND DEPRIVATION. E.Melamed,Y. Frucht*,M. Lemor* and J.Vidauri*. Lab. of Clin. Neurochemistry, Dept. of Neurology, Hadassah University Hospital, Jerusalem, Israel.

Dopamine (DA) turnover in retina is accelerated by photic stimulation.We investigated responses of rat retinal DA neurons to various effects of light exposure and deprivation.Rats,dark-adapted for 24hr, were killed in the dark or after being exposed to regular laboratory light for 5,15,30,60,or 120min. Retinal DA and DOPAC gradually increased up to 1hr with no further elevations thereafter.Groups of rats kept in a regular 12hr light-dark cyclic illumination (lights on at 6PM),were sacrificed at 2hr intervals begining at 6PM. Both,DA and DOPAC in retina markedly increased and remained elevated throughout the "lights-on" hours.They decreased and remained low during the"dark period".To determine whether the stimulatory effect of light on retinal DA neurons is mediated through photoreceptors, 24,35,or 70-day-old Royal College of Surgeons (RCS) rats with inherited progressive retinal dystrophy and age-matched controls were dark-adapted for 24hr and then exposed to light for 2hr. In controls,light exposure increased retinal DA and DOPAC levels in all age groups.Light stimulation enhanced retinal DA and DOPAC only in the 24-and 35-day-old RCS rats with functioning photoreceptors but not in the 70-day-old animals where all photoreceptors had been lost. Data suggest that intact photoreceptors are essential for light-induced activation of retinal DA neurons. To determine the effect of light deprivation on ontogenesis of retinal DA neurons,rats reared from birth in total darkness were killed in the dark 12,16,20,30,or 60 days postnatally.Age-matched controls kept in a regular light-dark cycle were killed at same ages after 24hr of dark adaptation.In controls,DA levels in retina increased up to 60 days after birth.In light-deprived rats, retinal DA levels failed to increase with age and were markedly smaller than those in controls.To determine the duration of postnatal light deprivation beyond which suppression of retinal DA levels becomes irreversible,rats were kept in total darkness for 16,20,or 30 days after birth and then transferred to normal light-dark conditions for periods up to 60 days.Controls were reared in normal lighting conditions or were totally light-deprived for 60 days postnatally.In rats grown in darkness and then changed to normal conditions, retinal DA levels were not lower but even higher as compared with controls.Sixty day-old rats raised from birth in a normal light-dark cycle were killed 7,14,30, or 60 days after being transferred to total darkness.Controls were killed after 24hr dark adaptation.In light-deprived rats, retinal DA gradually diminished and after 60 days was markedly lower than in controls.Findings suggest that postnatal light deprivation probably reduce synthesis and accumulation of DA and not the number of DA neurons in retina.

234.2 DOPAMINE AND EPINEPHRINE ARE CONTAINED IN SEPARATE AMACRINE CELLS IN RAT RETINA. M. Hadjiconstantinou*, A. Mariani*, P. Panula* and N.H. Neff. Lab. Preclinical Pharmacology, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032 and NEI, Lab. Vision Research, Bethesda, MD 20205.

There is now biochemical and pharmacological data supporting the presence of dopamine (DM)- and epinephrine (EPI)-containing neuronal elements in rat retina. We provide morphological evidence that they are contained in separate types of neuronal cells. Dopaminergic amacrine cells displaying bright, bluish-green fluorescence characteristic of catecholamines were seen in whole flat-mounts of the rat retina following fixation in an aqueous formaldehyde-glutaraldehyde (Faglu) mixture. After the systemic administration of pargyline, a monoamine oxidase inhibitor, some of the bright DM-containing cell dendrites in the inner plexiform layer were found to give rise to outwardly coursing processes which ramify in the outer plexiform layer indicating that some of the bright dopaminergic neurons are interplexiform cells. In addition, a third population of relatively weakly fluorescing amacrine cell bodies appeared. The color and intensity of their fluorophore suggested that they contain a catecholamine, perhaps EPI. This hypothesis was confirmed by the immunohistochemical localization of the EPI synthetic enzyme, phenylethanolamine-N-methyltransferase to amacrine cells. Our results indicate that three types of rat retinal neurons contain catecholamines. DM is present in a single type of amacrine cell and in interplexiform cells, EPI is contained in another type of amacrine cell.

234.3 RETINAL GABA NEURONS: IMMUNOCYTOCHEMICAL LOCALIZATION USING A NEW ANTISERUM AGAINST RABBIT BRAIN GLUTAMATE DECARBOXYLASE. Christopher Brandon. Department of Anatomy, Oregon Health Sciences University, Portland, Oregon 97201.

Much evidence exists to support the notion that γ-aminobutyric acid (GABA) is a neurotransmitter in at least some retinal neurons. One reliable method for identifying such cells is the immunocytochemical localization of glutamate decarboxylase (GAD), the enzyme responsible for the last step of GABA biosynthesis (see, e.g., Saito et al., PNAS 71, 269 (1974); Brandon et al., PNAS 76, 3557 (1979)). In order to examine such cells in detail with this method, a new antiserum has been produced against GAD from rabbit brain, and has been used for the localization of retinal GABA neurons in several species.

Rabbit brain GAD was partially purified by homogenisation and centrifugation, ion exchange and hydrophobic column chromatography, gel filtration, and gradient hydroxyapatite chromatography. This preparation, about 20% pure, was used for the immunization of several rabbits. One resulting multivalent antiserum was then used for the preparative immunoelectrophoretic production of a GAD immunoprecipitate according to Kroll and Andersen (J. Immunol. Methods 13, 125 (1976)). This GAD immunoprecipitate was then used in turn as an immunogen in a new rabbit. After several biweekly injections, an antiserum was obtained which formed a single precipitin arc on immunodiffusion or immunoelectrophoresis against a rabbit brain homogenate, and which precipitated GAD from crude homogenates (although it did not inhibit GAD enzymatic activity). By immunodiffusion, it cross-reacted with GAD from rabbit, rat, and goldfish.

This new antiserum has been used for the light-microscopic immunocytochemical localization of GABA neurons in the retinas of goldfish, catfish, turtle, chick, mouse, rat, rabbit, cat, monkey, and man. In all cases, GAD-immunoreactive processes were observed throughout the inner plexiform layer, although the patterns of lamination formed by these labeled processes were different in different species; in all species, some amacrine cell bodies were clearly labeled as well. In all non-mammalian retinas examined, sub-populations of horizontal cells were immunoreactive. Using this approach, the detailed synaptic connectivity of GABA neurons in the retinas of cat, rabbit, and monkey is currently under investigation.

Supported by USPHS EY-03886.

234.4 DEVELOPMENT OF GLUCAGON-LIKE IMMUNOREACTIVITY IN THE CHICK RETINA. M.Cilluffo,* T.Yamada,* and N. Brecha.
Center for Ulcer Research and Education, VA Wadsworth Medical Center and UCLA School of Medicine, Los Angeles, CA 90073

Previously we reported the presence of pancreatic type glucagon in the vertebrate retina. In our present studies we have examined the distribution of glucagon-like immunoreactivity (GLI) in developing and mature chick retina.

Retinas from staged chick embryos and young and adult chicks were immersion fixed in paraformaldehyde and processed according to standard immunohistochemical techniques. Using antisera against porcine pancreatic glucagon (N211 and GVI), GLI was found in at least 3 distinct populations of amacrine cells. The majority of cells present in all retinal regions had small, round somata and processes which primarily ramified in lamina 1 of the IPL. A second cell type had large, round somata and a single, short, thick primary process oriented parallel to the IPL. This primary process gave rise to multiple fine secondary processes extending to laminae 1, 3 and 5 of the IPL as well as to a single process that coursed to the ora serrata where it joined a fascicle of GLI processes. Medium sized cells were observed in peripheral retina and they gave rise to processes which joined the GLI fibers in the ora serrata. In the stage 39 (day 13) chick embryo retina, GLI was observed within "immature-appearing" somata lacking processes located in central retinal regions of the proximal INL. GLI containing processes were first observed in lamina 1 of the IPL at stage 40. By stage 43 (day 17), GLI was observed in all retinal regions and processes were observed in the ora serrata and laminae 1, 3 and 5 of the IPL as in the adult retina.

Radioimmunoassay of acid-ethanol extracts of retinas using pancreatic glucagon specific antiserum 30K revealed GLI concentration of 2.0pmol/g wet weight from stages 32 to 38. From stages 39 to 46 (hatching), retinal GLI rapidly increased from 2.6pmol/g ±1.6 to 15.3pmol/g±3.9. GLI peaked at day 1 (26.0pmol/g±6.1) and dropped to adult levels (14.9pmol/g±4.1) by day 3. Retinal extracts at stages 42, 46 and days 1, 2, 60, 90 were chromatographed on Sephadex G50. GLI was found to co-elute in a similar position to chicken pancreatic glucagon and synthetic porcine glucagon (Lilly).

These studies demonstrate that retinal GLI is present in distinct cell types which appear to develop simultaneously in the embryo. Tissue content of GLI matches the appearance of immunoreactive somata and processes. These studies suggest that the marked changes in retinal GLI at hatching is associated with changes in cellular GLI content and not the changes in numbers of GLI cells.

Supported by EY04067 and EY04172.

234.5 POSSIBLE CIRCADIAN VARIATION IN A PUTATIVE RETINAL NEUROTRANSMITTER. K.T. Keyser*, H.J. Karten, V.M. Cassone and M.Menaker.(Spon:J. Kleinschmidt). Dept. of Psychiatry, S.U.N.Y.at Stony Brook, N.Y. 11794, Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

The identification of putative transmitters and an understanding of their distributions and physiological roles in vivo remain central problems in neurobiology. The current wide availability of antisera directed against a variety of neuropeptides, enzymes and traditional transmitter candidates has greatly encouraged such studies.

The bird retina contains numerous classes of morphologically-distinct amacrine cells, many of which have recently been shown to be immunohistochemically distinct. For example, cells exhibiting Substance P-like reactivity in the pigeon retina are essentially identical to a cell type described by Cajal (Brecha & Karten, 1980).

We have begun investigation of the changes in levels of putative transmitters in amacrine cells of the pigeon as a function of time of day. Birds maintained on a 12:12 light-dark cycle were anesthetized and decapitated, the eyes infused with fixative, rapidly removed from the skull and the retinae processed for indirect immunoflorescence. Retinae from birds sacrificed at midnight showed dramatically increased staining of some amacrine cell types when compared to retinae from birds sacrificed twelve hours earlier. These included cells exhibiting enkephalin-like immunoreactivity (ELI), tyrosine hydroxylase-LI (THLI), and serotonin-LI (5-HT LI). In all three cases the cell bodies seemed more numerous and more extensive arboriztions were visible.

In some experiments, birds to be sacrificed at midnight were given a one hour exposure to room light before decapit ation and "noon"birds were kept in the dark for one hour. The retinae from these midnight birds again exhibited more intense staining in all three of the categories listed above. In addition to the enhanced 5-HTLI in amacrine cells of the midnight birds,there is a population of bipolar cells which was affected. The 5-HTLI bipolars appeared more numerous and their processes seemed more intensely stained in the midnight cases.

Preliminary biochemical data from chickens indicates about a twofold increase in measurable serotonin in the retinae of midnight birds (124pg/mg compared to 72pg/mg).

These observations raise the question as to whether these changes in levels and staining of putative transmitters are due to an actual cicadian rhythm. Experiments are currently being designed to test this hypothesis by using "free running" (i.e. under constant light or dark conditions) animals to determine whether or not the variation persists. Supported by EY 04796 to H.J.K. and AM 26972 to M.M.

234.6 ASPARTATE AMINOTRANSFERASE-LIKE IMMUNOREACTIVITY AS A MARKER FOR ASPARTATE/GLUTAMATE IN THE MONKEY RETINA. Judith L. Mosinger* (SPON: J. Erichsen). Dept. of Cellular & Developmental Biology, Harvard University, Cambridge, MA 02138.

Aspartate and/or glutamate have been proposed as transmitters in the vertebrate retina. The enzyme, aspartate aminotransferase (AAT), is involved in the metabolism of aspartate and glutamate. Previous studies have suggested that the antibody to AAT may serve as a marker for aspartergic/glutaminergic neurons (Altschuler, et al., PNAS, 78:6553, 1981). Peroxidase-antiperoxidase techniques were used for light and electron microscopic localization of AAT-like immunoreactivity in the cynamolgus monkey retina.

At the light microscopical level, a band of immunoreactivity is seen in the outer plexiform layer. This band is thick in the peripheral retina and sparse in the central retina, disappearing entirely near the foveal region. Electron microscopical analysis shows reaction product is localized to rod spherules, consistent with the gross regional distribution of AAT in the retina.

Two types of labeled cells are seen in the inner nuclear layer (INL): numerous somata in the position of bipolar cells and approximately 10-15% of the neurons on the proximal edge of the INL. AAT-like immunoreactivity is seen in both amacrine and bipolar terminals in the electron microscope. Labeled bipolar terminals are seen exclusively in the proximal half of the inner plexiform layer. They synapse mainly onto unlabeled and occasionally labeled amacrine processes. Labeled amacrine processes synapse upon and receive input from other amacrine processes and bipolar terminals.

A few large neurons in the ganglion cell layer also show AAT-like immunoreactivity in the light microscope. Their identity as ganglion cells or displaced amacrine cells is uncertain.

These results differ from those of similar experiments in guinea pig retina where cone and amacrine endings show AAT-like immunoreactivity but rod and bipolar terminals do not (ARVO Abstract, 22:247, 1982). These results support the hypothesis that aspartate and/or glutamate is a neurotransmitter in rods and a subpopulation of amacrine and bipolar cells in the cynamolgus retina.

234.7 EFFECTS OF DOPAMINE, FORSKOLIN, L-GLUTAMATE, Mg^{2+}, AND PYROGLUTAMATE ON GABA RELEASE FROM THE TELEOST RETINA. D. R. O'Brien* and J. E. Dowling. (SPON: M. Karnovsky), The Biological Laboratories, Harvard University, Cambridge, MA 02138.

We examined the rate of release of ^{3}H-GABA from intact carp and goldfish retinas using a "static perfusion" technique. Small, significant increases in the rate of GABA release were observed when the retinas were exposed to dopamine (DA) (10-100 µM); however, when free Ca^{++} was removed from the medium, the basal rate of GABA release was increased and DA became inhibitory. Forskolin, a non-specific stimulator of adenylate cyclase in intact cells, also inhibited GABA release in the absence of Ca^{++}. There was no significant effect of forskolin in the presence of Ca^{++}. L-glutamic acid (L-Glu) (1-10 mM) caused up to a 10-fold increase in GABA release. This effect of L-Glu was not significantly altered by removing Ca^{++} from the medium. In the presence of Ca^{++} DA did not significantly alter the effects of L-Glu; however in the absence of Ca^{++} a significant inhibition of the effects of L-Glu by DA was observed. These results suggest to us a model wherein DA stimulates Ca^{++}-dependent GABA release from one site and inhibits Ca^{++}-independent GABA release from another site via a cAMP mediated event.

Yazulla earlier reported (Invest. Ophth. Vis. Sci. 24 (Suppl): 40, 1983)that the effects of L-Glu in stimulating GABA release were significantly inhibited by 1 mM Mg^{++}. We have confirmed these observations. However, low concentrations of pyro-glutamate (pGlu) (10 µM) also inhibited the effects of L-Glu to the same extent as did Mg^{++}. Since neither pyroglutamate nor Mg^{2+} affected the basal rate of GABA release, these data raise the possibility that a retinal glutamic acid cyclotransferase converts Glu to pGlu in the presence of Mg^{++}. Such Mg^{++}-dependent cyclotransferase activity has been observed in a number of tissues, including CNS (Orlowski & Meister, The Enzymes 4: 123, 1971).

234.8 MONOCLONAL ANTIBODIES ASSOCIATED WITH H1 HORIZONTAL CELLS IN THE CARP RETINA. L. H. Y. Young* and J. E. Dowling. The Biological Laboratories, Harvard University, Cambridge, MA 02138.

Horizontal cells in the teleost retina have been classified into four subtypes with respect to their shape, location in the outer plexiform layer, specificity of synaptic connections and electrophysiological responses. The possibility that in addition to these differences there are distinct patterns of surface molecules among horizontal cell subtypes prompted our use of monoclonal antibodies as probes to further enhance our understanding of the cell surface properties of horizontal cells.

Balb c mice were injected with either carp retinal cell mixtures (approximately 10% horizontal cells) or purified horizontal cells obtained through velocity sedimentation Ficol gradients. Four hybridomas secreting antibodies specific to the outer plexiform layer in the carp have been raised. These hybridomas originated from 2 separate fusions, one from fusion of SP2/0 myeloma cells with spleen cells from a mouse immunized against gradient isolated horizontal cells and the other three from a mouse immunized against retinal cell mixture. All four monoclonal antibodies produce a very similar immunofluorescent staining pattern in the frozen sections of carp retina at the light microscopy level. The staining extends all along the outer plexiform layer and appears to be related only to the external (H1) horizontal cells. Of particular interest is the observation that the staining pattern is very similar to the outer plexiform layer dopamine fluorescence obtained with the Falck-Hillarp method. However, the staining is unaltered following 6-hydroxydopamine treatment which selectively destroys the terminals of the dopaminergic interplexiform cells. Our results thus suggest that the antibodies may be recognizing an element postsynaptic to the interplexiform cell terminals or some nearby molecule on the H1 horizontal cells.

234.9 ^{3}H-DOPAMINE RELEASE FROM THE INTACT CARP RETINA: EFFECTS OF K^{+}-IONS, 5HT AND (-)-3PPP. S. J. Dorison*, K. J. Watling* and J. E. Dowling (SPON: E. M. LASATER). Biological Laboratories., Harvard University, Cambridge, MA 02138.

The effects of K^{+}-ions, 5HT and (-)-3PPP have been investigated on ^{3}H-dopamine (DA) release from the dopaminergic interplexiform cells present in the carp retina. Dark-adapted carp retinas were removed and incubated for 15 min. in oxygenated Ringer's (pH 7.4) containing 0.2 µM ^{3}H-DA. Following a 10 min. rinse in DA-free Ringer's, retinas were placed, receptor side up, in a perfusion apparatus, such that each retina received a constant drip of Ringer's at a flow rate of ~2 ml./min. Samples of Ringer's were collected every minute and the ^{3}H-DA efflux determined by scintillation counting. Autoradiographic techniques, performed on pieces of retina processed according to the above protocol, revealed the presence of extensive labelling surrounding the horizontal cells in the outer plexiform layer (OPL), consistent with the uptake of ^{3}H-DA by the DA-ergic interplexiform cell terminals. Perfusion of retinal pieces with 1 min. pulses of Ringer's containing high concentrations of K^{+}-ions resulted in a large, dose-dependent efflux of ^{3}H-DA, with 30 mM K^{+}-ions evoking a 2-4 fold increase over basal ^{3}H-DA release. This response did not occur in Ca^{++}-free Ringer's, or in fish that had previously received intraocular injections of 6-hydroxydopamine, a treatment known to destroy selectively DA-ergic nerve terminals in the OPL. In agreement with the data of Kato et al. (J. Neurochem. 39, 493-498, 1982), both 5-hydroxytryptamine (5HT) and tryptamine induced a dose-dependent (1-300 µM) increase in ^{3}H-DA release when added to the perfusion Ringer's. However, contrary to the findings of the above workers, this response was neither Ca^{++}-dependent, nor blocked by 5HT antagonists, including cyproheptadine, methysergide and spiroperidol. These data raise the possibility that this 5HT effect is not mediated through a receptor specific mechanism located on DA-ergic terminals, but rather via an exchange mechanism. In contrast, (-)-3-(3-hydroxyphenyl)-N-n-propylpiperidine ((-)-3PPP), a putative presynaptic dopamine receptor agonist, did evoke a dose-related, Ca^{++}-dependent increase in ^{3}H-DA release. Furthermore, 100 µM (-)-3PPP greatly enhanced the response to 30 mM K^{+}-ions. These data raise the possibility that presynaptic dopamine receptors may modulate dopamine release in the carp retina. Various other retinal neurotransmitter candidates, including GABA, glycine, glutamate, aspartate, acetylcholine, enkephalin, thyrotropin releasing hormone and vasoactive intestinal peptide were all without effect either on basal or K^{+}-ion stimulated ^{3}H-DA release.

234.10 LIGHT MICROSCOPIC IMMUNOCYTOCHEMISTRY OF SOMATOSTATIN IN THE RAT RETINA. S.M. Sagar, P.E. Marshall* and D.M.D. Landis. Neurology Service, Massachusetts General Hospital, Boston, MA 02114.

Following paraformaldehyde fixation, Vibratome cross sections of rat retina were reacted with a commercially obtained antibody raised in rabbit to somatostatin-14 conjugated to bovine serum albumin. Triton-X100 was used to aid antibody penetration. The primary antibody was visualized using biotinylated second antibody and avidin-biotin-peroxidase complex. Control sections, incubated in primary antiserum preadsorbed with 10 ng/ml somatostatin-14, showed no selective staining of fibers or cell bodies.

Specifically stained cell bodies were found at the inner margin of the inner nuclear layer, some of which were seen to give off one or two thick processes passing into deep layers of the innner plexiform layer. In addition, a meshwork of fine, varicose somatostatin-reactive fibers was seen at the outer border of the inner plexiform layer. Occasionally, stained varicose fibers were seen running horizontally at the approximate midpoint of the inner plexiform layer as well as in deeper sublamina of that layer. Of particular note were extremely fine, varicose immunoreactive fibers which were seen to pass vertically or obliquely through the inner nuclear layer and bifurcate at its outer margin. Some of these fibers were continuous with short segments of immunoreactive, varicose fibers which were observed to run horizontally in the outer plexiform layer. Rare stained cell bodies in the ganglion cell layer with processes entering the inner plexiform layer were visualized, but their significance is uncertain in view of the high background staining of the ganglion cell layer.

These observations are consistent with rat retinal somatostatin being contained within diffuse amacrine cells. Some or all of these somatostatin-reactive cells are interplexiform cells. A cell type in the ganglion cell layer may also contain somatostatin. Recent immunocytochemical studies have localized tyrosine hydroxylase to interplexiform cells of the rat retina (Nguyen-Legos et al., Neuroscience Letters, 27 (1981) 255-259). The possibility of cohabitation of somatostatin and dopamine within the rat retina is therefore being investigated.

234.11 THE ORGANIZATION OF THE GANGLION CELL DENDRITIC GRIDS IN THE RETINA OF Astronotus. Roger P. Zimmerman, Departments of Neurological Sciences and Physiology, Rush University, Chicago, IL 60612.

The inner plexiform layer (IPL) of the retina of the cichlid fish Astronotus ocellatus exhibits two repeating anatomical patterns. The three-dimensional structure of these patterns has been studied using electron microscopy in combination with Golgi, Bodian, and horseradish peroxidase (HRP) labeling techniques.

The radial processes of the Müller glial cells form a roughly square array (nearest neighbor distance of 11.7 $\pm$ 0.2 micrometers, mean $\pm$ S.E.M.) in the plane of the retina.

The other repeating pattern is composed of ganglion cell dendrites, which form two gridworks of fibers that divide the thickness of the IPL into roughly equal thirds. These grids form two square lattices within the plane of the retina with a repeat distance of approximately 12 µm between nodes. The proximal (vitread) grid is rotated 45 degrees with respect to the distal (sclerad) grid.

Retrograde labeling of ganglion cell dendrites with HRP reveals that each grid has a substructure in the radial dimension. The distal grid extends from 20 - 45% of the inner plexiform layer thickness (roughly Cajal's sublamina 2), and the proximal grid extends from 55 - 85% of the IPL (sublamina 4 plus the adjacent halves of sublaminae 3 and 5). Both grids consist of two dense sublaminae rich in ganglion cell dendrites which surround a layer relatively poor in ganglion cell dendrites.

The majority of the individual ganglion cells which contribute dendrites to the grids have cell bodies (12 - 20 µm in diameter) in the ganglion cell layer, and multistratified dendritic arborizations which extend into both grids. In addition, there are large wide-field displaced ganglion cells which participate only in the distal grid.

The synaptic relationships of the ganglion cell dendrites within the grids and the relationship of the dendritic grid to the radial Müller processes will be discussed.

Supported by EY 03189, BRSG grant SO 7RR0547, and grants from Rush University.

234.12 EXPRESSION OF SUBSTANCE P- AND VIP-LIKE IMMUNOREACTIVITY BY RAT RETINAL NEURONS IN CELL CULTURE. M. Fukuda*, H.H. Yeh and D.G. Puro (Spon: H.H. Hess). Laboratory of Vision Research, National Eye Institute, NIH, Bethesda, Maryland 20205.

Both substance P and VIP have been localized by immunohistochemistry to amacrine cells of the vertebrate retina. Despite knowledge of their localization, much remains to be learned about the retinal cells which contain these neuropeptides. Since cell culture provides a potentially useful approach to the study of neuropeptide-containing nerve cells, retinal cells of perinatal rats were maintained in cell culture and examined for immunoreactivity.

Cultures containing dissociated retinal cells were prepared as previously described (Puro et al., 1982). At various times after the plating of the retinal cells, the cultures were initially incubated for 24 hr at room temperature with substance P or VIP antiserum at dilution of 1:500-1:1000. The cultures were then incubated for 1 hr at 37^{o} C with FITC-labeled antiserum diluted 1:100. Cultures were examined using phase contrast and fluorescence microscopy.

Substance P- and VIP-like immunoreactivity was detected within two weeks of culture. Both substance P- and VIP-positive cells had somas of 7 to 10 µm in diameter and could be of a variety of morphological types. Neurons staining for substance P- or VIP-like immunoreactivity were usually in close proximity to non-staining retinal cells. Only a relatively small percent of the neurons in our cultures displayed either substance P- or VIP-like immunoreactivity.

The results suggest that substance P and VIP are present in a number of morphological subtypes of retinal neurons in our cultures. This cell culture system should be useful in further studies of the role of substance P and VIP in the mammalian retina.

234.13 SPECIFIC TYPES OF HORIZONTAL AND GANGLION CELL IN CAT RETINA STAINED BY ANTISERUM RAISED AGAINST ALPHA-MSH. R. Stone, A. Laties and P. Sterling. (Spon: T.L. Davis). Depts. of Anat. & Ophthalmol., Univ. of Penna. Sch. of Med., Phila., PA 19104.

The cat retina contains two morphological forms of horizontal cell and 23 morphological forms of ganglion cell. Each form studied so far has a characteristic cytology, set of connections, and physiological properties, but no characteristic chemical features have been identified. We report here that an antigen identified by its immunoreactivity with antiserum raised against alpha-MSH is present in horizontal and ganglion cells of particular types. Retinas from adult cats fixed by perfusion with 4% paraformaldehyde solution, 0.1 M lysine HCl and 0.01 M Na periodate in 0.1 M phosphate buffer, were sectioned radially and tangentially at 10um on a cryostat. Antibody to alpha-MSH (Immunonuclear) was applied in dilutions 1:250 and visualized by means of FITC fluorescence and by HRP reaction product (avidin-biotin method). One-third of all horizontal cells stained. The neurons had thick dendrites and the appearance in tangential section of starfish; they formed a regular array with an average nearest neighbor distance of 66±14 um. Stained horizontal cells observed in the electron microscope had indented nuclei and clusters of clear vesicles that formed halos around a few smaller, dense-core vesicles. These neurons correspond in form, ctyology and distribution to the type A horizontal cell described by Boycott, Wassle, Kolb, et al. A subpopulation of the largest ganglion cells was also stained. These had radiating dendrites restricted to sublamina a of the inner plexiform layer suggesting that they are alpha (Y)-off ganglion cells. Consistent with this, we observed large axons stained in the optic tract and stained terminal arborizations in the lateral geniculate A-laminae and in the superficial gray layer of the superior colliculus. Some small ganglion cells with various morphologies also were stained. All had dendrites arborizing in sublamina a, suggesting they represent several types of off cell. Staining was not observed when the antiserum had been preincubated with 50um synthetic alpha-MSH. We have observed neuronal staining in the retina of many vertebrate species using this antiserum, but other well-characterized anti-α-MSH antisera have yielded negative histochemical or radioimmunassay results when applied to these retinas (unpublished observations). Whether the antigen identified in the current experiments is actually alpha-MSH or a cross-reacting molecule is uncertain. It does seem clear, however, that the antigen is contained in the A and not the B horizontal cells and in the alpha (Y)-off and not the alpha (Y)-on ganglion cell. This specific chemical feature thus contributes further to the definition of these cells as fundamental types in cat retina.

234.14 MONOCLONAL ANTIBODIES MARK SPECIFIC TYPES OF AMACRINE VARICOSITY IN CAT RETINA. P. Sterling and L.A. Lampson. Dept. of Anatomy, Univ. of Penna. Sch. of Med., Phila., PA 19104.

The neural processes belonging to 56 types of neuron (23 ganglion, 22 amacrine, 10 bipolar, 1 interplexiform) are intermingled in the inner plexiform layer (IPL) of the cat retina. Each process lies within microns of all the other 55 types but makes synaptic contact at specific loci with only a small subset of these. One way to account for this extraordinary specificity is to postulate the presence of synapse-specific "recognition molecules" on neuronal membranes at sites of synaptic contact. Here we describe 3 monoclonal antibodies whose binding in the IPL is restricted to specific populations of amacrine synaptic varicosity. A panel of monoclonal antibodies (MoAbs) was raised against homogenates of adult cat retina and screened, using the PAP technique, against 50um Vibratome sections of cat retina fixed by perfusion with 2% paraformaldehyde-2% glutaraldehyde. Of 41 cultures screened, 19 stained neurons and none stained Muller cells or glia. Many of the MoAbs stained the somas of amacrines, bipolars, and ganglion cells in different proportions. There was no Golgi-like staining of their arborizations in the IPL, such as observed with antibodies to peptides; instead there was intense staining of certain varicose structures in the IPL. The varicosities stained by three different MoAbs were studied by electron microscopy of serial sections. The varicosities were stained in both membrane and cytoplasm and proved to be the synaptic structures of amacrine processes. Ganglion cell dendrites, bipolar axons and the strands interconnecting amacrine varicosities were unstained. In sublamina b of the IPL, which we analyzed in detail, the stained varicosities made reciprocal contact with the rod bipolar axon at about half of the dyads. The AII amacrine process, which is the other member of the dyad, was never stained, nor were the non-reciprocal amacrines presynaptic to the rod bipolar at sites other than the dyad. The reciprocal varicosities belong to two types of GABA-accumulating amacrine (A_{13} and A_{17}). It is probable, since each antibody stained the reciprocal element at only about half the dyads, that each stains the varicosities of only one amacrine type. Whether or not the determinants recognized by these MoAbs belong to "recognition molecules", it does appear that the synaptic membrane of one specific neuron type can be distinguished immunologically in the presence of many others. Supported by NEI EY00828 and NINCDS NS16552.

234.15 GANGLION CELL MORPHOLOGY OBSERVED BY RETROGRADE HRP-FILLING IN FLAT MOUNTED RETINA. F.Scalia and V. Arango*. Dept. of Anatomy and Cell Biology, Downstate Medical Center, Brooklyn, N.Y. 11203.

The axons, dendrites and somata of retinal ganglion cells were selectively impregnated by a HRP-reaction product in flat mounted retinas of the frog, Rana pipiens, following application of the tracer to limited incisions in the intraorbital segment of the optic nerve. The small size of the nerve incisions permit the dendritic morphology of relatively isolated ganglion cells to be examined, and to be correlated with soma size, axon diameter and retinal locus. Preliminary observations indicate the presence of cells resembling the α, β and γ cells of the cat retina by virtue of their dendritic morphology, without reference to size. Most cells have roughly circular dendritic fields, although many α cells show strongly elliptical field contours, and apparent γ cells can be found having nearly linear arborizations. Soma diameters vary from 6-20 μm, and dendritic fields vary from 45-900 μm in the current sample. Further quantitation will be provided. (Supported by NSF grant BNS 79-23788.)

234.16 CELL DIFFERENTIATION IN THE RETINA OF THE SEA LAMPREY, PETROMYZON MARINUS. K. Rubinson and H. Cain.* Dept. Physiol. and Biophysics, NYU Med. Ctr., New York, NY 10016.

Our earlier report ('77) indicated that the central zone of the larval retina was histologically differentiated with disc-bearing outer segments. However, no evidence of a photolabile pigment or of an ERG could be demonstrated until the peripheral retina showed a similar differentiation late in transformation. This report describes that histological development of the peripheral retina using Golgi and toluidine-blue methods.

The youngest lamprey (Group I larvae) have only a narrow rim of peripheral retina surrounding the central zone and this is composed of a dense lamina of neuroblastic cells, each with cytoplasmic extensions to the inner and outer limiting membranes. Group II larvae show the beginning of a cell-sparse lamina between the dense lamina and the vitreal surface and the enlargement of the retinal surface area of the peripheral retina. Ganglion cells can be identified within the cell-sparse lamina and Muller cells within the neuroblastic mass.

In Group III, the cell-sparse lamina extends almost to the rim of the retina with the ganglion cells lying within the inner plexiform layer and vitread to the layer of optic nerve fibers. The cell-dense lamina has separated into a darkly-stained outer portion and a predominantly lightly-stained inner portion. Based upon Golgi impregnations, the inner portion contains Muller cells, amacrine cells, ganglion cells and, in its outer limits, immature horizontal cells. Undifferentiated neurons, corresponding in location to the darkly-staining cells, characterize the outer portion and a lamina within the inner portion.

As the animals enter transformation, the eye enlarges markedly (Stage I of Manion and Stauffer, '70). Although the histological appearance of the peripheral retina is similar to that in Group III, its area also increases markedly while the extent of the central zone is unchanged.

Later transformers and adults differ from the above description in the appearance of photoreceptors with inner and outer segments and an outer plexiform layer. Although the outer segments may be short at first and the retinal histology is similar to that attained by the central zone years before, this development seems to be correlated with the appearance of the ERG and manifestation of a photolabile pigment.

Supported, in part, by NS15252.

234.17 **SIX-HYDROXYDOPAMINE-INDUCED DOPAMINE DEPLETION IN FROG RETINA**
L. Erinoff, M.C. Citron, N. Brecha, D. Rickman*. Neurology Res., Childrens Hospital of Los Angeles, Los Angeles, CA 90054 and CURE, VA Wadsworth Medical Center, Los Angeles, CA 90073.

In order to selectively destroy dopaminergic amacrine cells in the frog retina (Rana pipiens), 6-hydroxydopamine (6-HDA), 100µg in 5µl saline-ascorbate, was injected intravitreally into one eye; vehicle injection into the other eye served as control. After two weeks eyes were excised and ERGs were recorded from an eyecup preparation. Following recording, retinal dopamine (DA) and serotonin (5HT) concentrations were determined by HPLC with electrochemical detection.

DA content of the treated retinas was reduced by 91% (6-HDA treated 1.44±0.51 ng/mg protein, vehicle control 16.06±1.99 ng/mg) while 5HT levels were unchanged (treated 76.12±10.14 ng/mg protein, control 67.44±7.30 ng/mg). The ERG b-wave of the treated retinas showed an increased latency of 49.25±23.7 msec ($p < .025$) relative to the vehicle control.

An additional group of retinas was studied immunohistochemically for both tyrosine hydroxylase-like (TH) and 5HT-like immunoreactivity seven days after 6-HDA treatment. Normal, 6-HDA treated, and vehicle control retinas were fixed in 4% paraformaldehyde in 0.1M phosphate buffer, washed, and processed simultaneously according to standard immunohistochemical procedures. No TH-like immunoreactive staining was observed in retinas treated with 6-HDA, while 5HT-like immunoreactivity remained unchanged. In both normal and vehicle control retinas, the majority of TH-like immunoreactive somata were located in the inner nuclear layer (INL) at the border of the INL and inner plexiform layer (IPL); an occasional somata was also located in the ganglion cell layer. A dense, continuous band of varicose processes was located in lamina 1 of the IPL adjacent to the INL. A second discontinuous band of processes was present in lamina 5 of the IPL. Scattered processes were also present in laminae 2-4 of the IPL. TH-like immunoreactivity was not observed within the ganglion cell axon layer, distal INL, outer plexiform layer, or outer nuclear layer. Serotonin-like immunoreactivity was observed in a seemingly distinct amacrine cell population; 5HT immunoreactive somata were distributed in the INL at the IPL border and their processes were distributed to all laminae of the IPL.

Our results demonstrate biochemically and histochemically that it is possible to deplete dopaminergic amacrine cells selectively in the frog retina. Removal of these cells is correlated with an increased latency in the b-wave of the ERG.

Supported in part by NIH grants EY04711, EY04304, and EY04067.

234.18 NEUROTENSIN CONTAINING AMACRINE CELLS IN THE RETINA OF A TURTLE (PSEUDEMYS SCRIPTA): AN ULTRASTRUCTURAL AND BIOCHEMICAL STUDY.
W.D. Eldred and R.E. Carraway*
Dept. of Biology, Boston University, Boston, MA 02215
Dept. of Physiology, University of Massachusetts Medical School, Worcester, MA 01605

Antisera directed against immunoreactive neurotensin (INT) were used to specifically label two amacrine cell types in the turtle retina, both of which arborized within strata 3 and 4 of the inner plexiform layer. One of these cell types with INT had large elongated cell bodies which gave rise to several long tapering processes. Using peroxidase techniques at the ultrastructural level, the immunoreactivity was primarily confined to 1000 A vesicles, with little diffuse cytoplasmic reaction product. These large neurons received both conventional and ribbon synaptic contacts, and made conventional synaptic contacts containing unlabeled synaptic vesicles (525 A in diameter) onto other cells. The second cell type with INT had smaller rounded cell bodies which gave rise to a few short delicate branching cell processes. The cytoplasmic reaction product in these cells was very intense and obscured much of the intracellular detail. This cell type also received both conventional and ribbon synaptic contacts, and made conventional synaptic contacts containing unlabeled synaptic vesicles (600 A in diameter) onto other cells.

Biochemical studies were done to determine the identity of the INT localized in the ultrastructural studies. Isolated retinas were sonicated in 10 volumes of 0.1 N HCl on ice, and the extracts were examined for the presence of INT using four region-specific antisera. The results indicated the presence of substances (8-10 pMoles/g) resembling the C-terminal region of neurotensin, but differing at the N-terminal portion. During HPLC on micro-Bondapak C18 two major peaks of INT were obtained, one of which chromatographed near the position of turtle neurotensin obtained from the small intestine.

The combination of the ultrastructural and biochemical results suggests that a peptide, which closely resembles neurotensin found in gut, coexists with another neurotransmitter within these amacrine cells.

This work was supported by EY04785 to WDE and AM28565 to REC.

234.19 NEUROTRANSMITTER LOCALIZATION IN THE SKATE RETINA.
B. Ehinger, A. Bruun* and V.M. Sytsma*. Department of Ophthalmology, University of Lund, Lund, Sweden.

The retina of the skate (Raja clavata, R. radiata, and R. oscellata) was studied by autoradiography following intraocular injections or incubations with (^{3}H)-GABA, (^{3}H)-isoguvacine, (^{3}H)-glycine, (^{3}H)-dopamine or (^{3}H)-5-hydroxytryptamine. Fluorescence immunohistochemistry was also used to demonstrate the endogenous content, accumulation, and retention of 5-hydroxytryptamine.

The (^{3}H)-GABA was taken up by glia, and (^{3}H)-isoguvacine failed to appreciably label any neurons. (^{3}H)-Glycine was accumulated by amacrine cells, possibly of two subtypes. The (^{3}H)-dopamine was taken up by some few amacrine cells. Both autoradiography and immunohistochemistry showed 5-hydroxytryptamine to be efficiently accumulated by two types of cells in the inner nuclear layer: ON bipolar cells and some amacrine cells. The bipolar cells contained far less endogenous 5-hydroxytryptamine than the amacrine cells did.

The results show the localization of presumed glycinergic, dopaminergic and indoleaminergic neurons. They also show that there are two fundamentally distinct types of indoleamine neurons, a bipolar cell type with a low and an amacrine cell type with a high content of 5-hydroxytryptamine.

234.20 CYTOCHEMICAL LOCALISATION OF 5'-NUCLEOTIDASE IN THE RAT RETINA.
G.W. Kreutzberg and S.T. Hussain*. Max Planck Institute for Psychiatry, D-8000 Munich, F.R. Germany.

We have previously described the activity of 5'-nucleotidase (E.C.3.1.3.5.) in the brain to be associated predominantly with plasma membranes of glial cells (Kreutzberg et al., 1978, Brain Res. 158: 247). When studying the distribution of this enzyme in the retina of the rat we similarly found the enzyme to be very prominent on the glial cells of the Müller type. There is, however, an asymmetric distribution of this ecto-enzyme insofar as only those Müller cell processes extending into the external layers show 5'-nucleotidase activity (Kreutzberg and Hussain, 1982, J. Neurocytol. 11: 53). Further studies have revealed activity in the clefts of the complex synapses formed by the rod spherules, the horizontal and the bipolar cell terminals. In this location no glial elements are present. Thus, the enzyme must be of neural origin. Activity is also seen as an ectoenzyme on the axolemma of receptor fibers. In the rod inner segment strong reaction product is located intracellularly. In the rod outer segment the enzyme appears to be located only on the cytoplasmic side of the disc membrane and not intradiscally. Retinal pigment cells are rich in 5'-nucleotidase. Their microvilli accompany the tips of the receptor cells and show enzyme activity in an ecto position. A role for 5'-nucleotidase is possible in the metabolism of guanylate and adenylate nucleotides both of which are important for visual transduction processes.

234.21 ISOLATED, ARTERIALLY PERFUSED HUMAN EYE. M.L. Anderson, R.L. Purple, D. Burkhardt, J. Wirtschafter and T. Kraft. U of Mn. Med. Sch., Depts. of Physiology, Ophthalmology and Psychology.

Preliminary experiments have shown that ERGs recorded from brain dead, potential, multiorgan donors, are almost always indicative of a viable eye, despite absence of blood flow to brain tissue and lack of any brain stem reflexes, as prescribed by the U. of Mn. Dept. of Neurosurgery's definition of clinical brain death. Two of these potential organ donors actually had normal ERG's despite having been clinically brain dead and on a respirator, for more than 3 days.

Necessary approval for harvesting one eye for physiological research from such organ donors has been made through the U. of Mn. Human Subjects Volunteer Committee, the U. of Mn. Hospitals, and the Depts. of Ophthalmology, Neurosurgery, Surgery, and the Multiple Organ Donor Program. A portable perfusion apparatus has been constructed and tested successfully on cat eyes.

Initial results have been obtained from one human eye which was successfully perfused for over 5 hours. Due to other surgical procedures done on the donor, blood pressure had fallen to zero before surgical removal of the eye began. Artificial perfusion with an enriched tissue culture perfusion medium was established through cannulation of the ophthalmic artery approximately 40 minutes after blood pressure had fallen to zero. Initially, only a small a-wave could be recorded. Within 3-4 hours, at a perfusion rate of 4 ml/mn, the scotopic ERG had recovered to normal amplitudes (a-waves, b-waves and b-wave oscillations). These initial results indicate a rather remarkable ability of the eye to recover from prolonged anoxia. They also indicate the potential feasibility for direct research on the human eye.

This study has been supported by the Minnesota Medical Foundation and by the Minnesota Lions Eye Bank and Children's Fund. We thank Jane VanHook and the Multiple Organ Donor Program for their cooperation.

234.22 THE INVOLVEMENT OF CHLORIDE IONS IN HORIZONTAL CELL FUNCTIONING IN FISH RETINA. M.B.A. Djamgoz and P.J. Laming*. Dept of Pure and Applied Biology, Imperial College, London SW7 2BB, UK.

Intracellular measurements of chloride activity in fish and amphibian retinae showed that luminosity type horizontal cells (HC's) contain higher levels of Cl^- than necessary for passive distribution i.e. the chloride equilibrium potential, $E_{Cl} > E_m$, the membrane potential in the dark (Djamgoz and Laming, 1983; Miller and Dacheux, 1983). This raises the possibility that Cl^- are involved at an excitatory synaptic input to HC's, and in the mudpuppy retina, Miller and Dacheux (1983) suggested that Cl^- are indeed the ions responsible for generating the photoreceptor-driven, light-evoked hyperpolarizing responses of HC's. In cyprinid fish retina HC's receive excitatory synaptic inputs from the photoreceptors (PC's) and the interplexiform cells (IC's). Since the latter are known to use dopamine as their transmitter (Dowling and Ehinger, 1975), we tested the possibility that Cl^- are gated by dopamine at the IC → HC synapse.

The intracellular choride activities (a^i_{Cl}'s) and membrane potentials of 41 L_1(H1) type HC's (8 retinae) were measured simultaneously using double-barreled Cl^--sensitive microelectrodes employing the Corning exchanger 477315 in the isolated retinae of a common cyprinid fish, roach, *Rutilus rutilus*. Retinae were specifically not superfused with artificial salines so 'normal' ionic activities could be measured; 10 μM dopamine and 1 mM sodium ascorbate were applied from an atomizer system (Djamgoz, 1983). In control retinae $E_{Cl} - E_m$ had an average value of + 12 ± 2 mV. Following treatment with dopamine, however, HC's depolarized by an average of 20 mV, and the $E_{Cl} - E_m$ difference was reduced to − 0.7 ± 0.3 mV. All values are given as means ± SE's.

Thus, for HC's treated with dopamine, $E_{Cl} \simeq E_m$ thereby suggesting that Cl^- can permeate through dopamine-sensitive channels, presumably at the IC → HC synapse. If so, sodium ions, which are also required for generating light-evoked HC responses in fish retinae (Kaneko and Shimazaki, 1975), are probably involved at the PC → HC synapse.

REFERENCES

Djamgoz, M.B.A. (1983). *J. Neurosci. Methods* (in press).
Djamgoz, M.B.A. and Laming, P.J. (1983). *J. Physiol.* (in press).
Dowling, J.E. and Ehinger, B. (1975). *Science* 188, 270-273.
Kaneko, A. and Shimazaki, H. (1975). *J. Physiol.* 252, 509-522.
Miller, R.F. and Dacheux, R.F. (1983). *Vision Res.* 23, 399-411.

234.23 WAVELENGTH DEPENDENCE OF RESPONSES AND LIGHT ADAPTATION OF GOLDFISH H1 HORIZONTAL CELLS. R.P. Malchow and S. Yazulla. Department of Neurobiology and Behavior, SUNY Stony Brook, NY 11794.

Anatomical studies reveal that H1 horizontal cells of the goldfish retina contact all three types of cones. Proposed models suggest that H1 cells receive input from red cones and feed back onto red, blue and green cones. Electrophysiological evidence suggests that, while red cones dominate the response of H1 cell, they do not constitute its only input. We wished to study this question in greater detail, and to examine the effects of steady monochromatic backgrounds on the response properties of this cell.

Intracellular recordings were obtained from H1 cells in the isolated retina. Full field 651nm and 501nm stimuli produced responses which had different waveforms at equivalent response amplitudes. Dim 651nm stimuli produced very square responses which returned quickly to the dark adapted resting potentials at light offset. 501nm stimuli resulted in responses which reached a peak, then slowly decayed towards the dark resting potential despite the continued presence of the light. Light offset often resulted in a depolarizing overshoot which took several seconds to return to the dark resting potential. 651nm and 501nm stimuli also produced response vs. intensity (RvI) curves which differed in shape.

RvI curves for dark adapted H1 cells were broad, even when stimuli were short in duration. For .1sec 651nm stimuli, these cells spanned 4.6 log units of intensity in going from 1% to 99% of their maximal response (Vmax). Using a substitution paradigm, light adaptation with steady 651nm backgrounds produced a narrowing of the RvI curves leaving Vmax unaltered: a background of 13.6 log photons/sec.cm^2 resulted in an RvI curve spanning only 2.5 log units of intensity. Backgrounds produced a shift of the RvI curves to the right along the intensity axis. 501nm backgrounds resulted in enhanced responses to .1sec 651nm test flashes, as well as increasing Vmax and narrowing the RvI curves. These effects could be seen even when the 501nm background had small effects on the steady state potential of the cell. The potentiating effect was the same regardless of whether the 651nm test stimulus was presented as a 2mm spot, a 2mm i.d. x 3mm o.d. annulus, or a full field flash. This indicates that the effect of 501nm light is not mediated by the dopaminergic interplexiform cell, since the addition of dopamine reduces the receptive field size of H1 cells.

These data support the contention that H1 horizontal cells have inputs besides red cones and dopaminergic interplexiform cells. This input is unlikely to be due to rods, for the effects of 501nm stimuli begin at an intensity at which the rods saturate.

(Supported by NIH grant EYO 1682 to S.Y.)

234.25 A POWER LAW INTENSITY-RESPONSE RELATIONSHIP IN TURTLE CONES. V. Pluvinage* and D.G. Green, Univ. of Michigan, Ann Arbor, MI.

Using eyecup preparations, we recorded intracellularly the voltage response of red cones in Pseudemys scripta turtle retinae to a slit (width=5 um) or a spot (diam.=7 um) of light (λ=650 nm) flashed (dur.=20 msec) at different positions in the receptive field. The intensities used ranged from 10^4 to 10^7 photons/um^2·sec.

In a first experiment, the responses due to two small stimuli, one centered on the cone and the other one 30 um away, were analyzed using a method of excitation summation similar to the one first introduced by Easter in 1968. First, the intensity of the centered stimulus was adjusted so that it gave the same response amplitude as the displaced stimulus. Then they were flashed together, and the amplitude of the combined response was measured. The intensity of the centered stimulus was increased so that the central response was equal in amplitude to the combined response. This process was repeated for different intensities of the displaced spot to determine the intensity coding for the central stimulus. This was a power function with an exponent 0.48 (0.46 to 0.5, N=5) over a range of 2 log units. In a second experiment, we measured the receptive field in two different ways. For each position of the slit in the receptive field, we determined 1) the response amplitude to a fixed intensity and 2) the light intensity needed to elicit a fixed response amplitude. Both response amplitude and sensitivity decayed exponentially with distance from the impaled cone. Surprisingly, the two space constants were very different (29 um and 15.1 um respectively, N=4). The average ratio was 1.92. For one cone, the receptive field was asymmetrical: on one side the response space constant was 37 um, on the other 24 um. The sensitivity space constants were 18.5 um and 12.6 um respectively, so that, on each side, their ratio was close to 2:1. These and other experiments are consistent with a response of the form: $V(x,I) = c \cdot I^{\frac{1}{2}} \cdot \exp(-x/L)$, where c is a constant, I is the light intensity, x is distance and L is the response space constant.

This finding may be related to experiments on ganglion cells in goldfish and cat that suggested the existence of a power law relation with an exponent of 0.5 between light intensity and some early stage of signal processing in the retina (Easter, 1968; Levine and Abramov, 1975; Enroth-Cugell & Harding, 1980). We now have evidence that such a relationship exists in a coupled cone in an intact retina. We will discuss a possible explanation of this result.

(Supported by Grant EY-0379)

234.26 AN ON-OFF AMACRINE CELL TYPE IN THE CAT RETINA. Helga Kolb and Ralph Nelson*. Department of Physiology, University of Utah School of Medicine, Salt Lake City, Utah 84108, and Laboratory of Vision Research, National Eye Institute, Bethesda, Maryland 20205.

Intracellular recording and staining with horseradish peroxidase (HRP) has revealed a new amacrine response type in the cat retina. Although common in lower vertebrate retinas, the ON-OFF response type is rarely encountered in the cat retina, where most amacrine cells give either a sustained depolarization or a sustained hyperpolarization (A13, A17, A4, A6). Transient ON (AII) and transient OFF (A8) types have also been observed. It was, thus, of extreme interest to discover an amacrine cell in this species that responded to a broad-field stimulus with a transient depolarization at both light onset and offset.

In whole-mount view, the HRP injected cell exhibited the morphology of the A19 cell described in our Golgi study (Kolb et al., 1981). The cell has a large cell body (12 x 14 um) and a large monostratified dendritic tree covering an area of 600 x 750 um. Two major dendrites leave the cell body, descend to S2 of the IPL and there arborize into a radiate dendritic tree of linear, sparsely-branching dendrites. The major dendrites are of large caliber, intermittently varicose or beaded and bear a few small spines and appendages. Electron microscopy of the HRP stained example indicates that the major input to the linear dendrites is from amacrine cells while amacrine and cone bipolar cell input occurs onto the spines and appendages. In cross section the larger primary dendrites are seen to be filled with neurotubules thereby giving A19 cells a characteristic and recognisable cytoarchitecture within the IPL. In fact neurotubule-filled profiles of similar dimensions and synaptic characteristics have been seen to make gap junctions with like amacrine profiles. These in turn are presynaptic to another amacrine cell type, A17, known to be involved in the rod system of the cat retina.

In lower vertebrate retinas (e.g. fish and amphibia) it has been suggested that ON-OFF amacrines are bistratified and aquire their ON and OFF input from the differently signed responses of bipolar cells branching in the two sublaminae of the IPL. The ON-OFF A19 amacrine of the cat retina receives input primarily from other amacrines. The only bipolar input is from cone bipolars of sublamina a known to be OFF center. Based on our present knowledge then, amacrine cells will have to be invoked to explain the ON-OFF response of the A19 cell of the cat retina.

234.27 SPATIAL DISTRIBUTION OF INPUT FROM DEPOLARIZING CONE BIPOLARS TO DENDRITIC TREE OF ON-CENTER ALPHA GANGLION CELL M. A. Freed and P. Sterling. Dept. of Anat., Sch. of Med., U. of Pa., Phila.,Pa., 19104

Excitation at light on is conveyed to the the on-center beta (X) ganglion cell of the cat retina by bipolars of type CBb_1. Type CBb_1 is apparently depolarizing and excitatory with a narrow field and an antagonistic surround. Each of three CBb_1s contacts the beta cell at least 50 times, ending on dendrites of all orders spread widely over the dendritic tree. The distribution of CBb_1 inputs to the on-center alpha (Y) ganglion cell showed a pattern that was strikingly different.

An on-center alpha cell within 2 degrees of the center of the area centralis was filled by retrograde transport of HRP injected into the optic disk. The cell, stained in a Golgi-like manner by reaction with DAB, was cut into a series of 420 tangential sections each 90 nm thick. We have reconstructed about 10% of the dendritic tree, an area 30 um wide that extended 60 um from the soma. Included were two primary dendrites and all daughter branches out to the sixth order.

Ten CBb_1s were found in the area studied. These formed a regular array, with an average nearest neighbor distance between axonal stalks of 10±1 um. The bipolars made an average of four contacts to the alpha cell dendritic shafts and to thin (.3 um) spines, which often invaginated the bipolar axon terminal.

The contacts from each CBb_1 were localized on the alpha dendrites to one side of the nearest branch point, sometimes contacting two daughter branches that resulted from a bifurcation. In such cases the parent dendrite was not contacted by the same bipolar. Thus, contacts from each CBb_1 were restricted to a single order of dendrite. Contacts from one CBb_1 were never interpolated with those of another.

Two other types of cone bipolar contacted the alpha cell, mostly on proximal dendrites. One possessed large diameter (3 um) dark varicosities in stratum 5. The other type had moderately dark, smaller swellings in stratum 4. Five rod bipolars contributed one contact each; these were on spines and dendritic shafts of the first to the fourth order.

We extrapolate from the area studied that the whole alpha cell receives input from about 100 cone bipolars of type CBb_1, each contributing about four inputs at a distinct electrotonic distance from the soma. The on alpha (Y) cell dendritic field is known to be cospatial with the on center of the receptive field. Apparently, excitation evoked by stimulation of the receptive field center comes from an array of CBb_1 bipolars, arranged such that excitation at progressively more peripheral loci in the receptive field is from bipolars at progressively greater electrotonic distances from the soma.

234.28 CHANGES IN THE RECEPTIVE FIELD PROPERTIES OF GANGLION CELLS IN THE RABBIT RETINA WITH DOPAMINE ANTAGONISTS. R. J. Jensen* and N. W. Daw (SPON: Y. Fukami). Dept. of Physiology and Biophysics, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Much evidence has accumulated implicating dopamine as a neurotransmitter in the retina. Very little is known however of the functional role of dopamine in visual processing, particularly in the mammalian retina. We decided therefore to investigate the effects of dopamine antagonists on the receptive field properties of retinal ganglion cells in the intact, anesthetized rabbit. Extracellular recordings were taken of ganglion cell responses to spots and annuli of light before, during and after drug infusion of a dopamine antagonist in the arterial system supplying the eye.

Dopamine antagonists haloperidol, fluphenazine, and cis(Z)-flupenthixol were used and found to produce similar changes in the receptive field properties of ganglion cells. All OFF-center brisk cells showed reduced spontaneous activity and reduced surround ON responses upon infusion of a dopamine antagonist. The drug effect on ON-center brisk cells depended upon the specific cell type. ON-center brisk-sustained cells showed a marked increase in spontaneous activity and a change in the center-surround balance in favor of the surround, whereas ON-center brisk-transient cells showed little change in spontaneous activity and a change in the center-surround balance in favor of the center. ON-OFF directionally selective cells showed reduced ON responses to spots of light and reduced leading-edge responses to moving bars of light. In addition, the dopamine antagonists delayed ON or OFF light-evoked responses of ganglion cells frequently by tens of milliseconds. Recovery from the drug effects took 1-3 hours.

In addition to D-1 (adenylate cyclase linked) receptors, the rabbit retina appears to have D-2 receptors (Dubocovich, M. L. and Weiner, N., J. Pharmacol. Exp. Ther., 219:701, 1981). The effects described above appear to be due to blockage of D-1 receptors rather than D-2 receptors since neither metoclopramide nor S-sulpiride, two specific D-2 antagonists, had much effect.

Supported by NIH Grants EY07057 and EY00053.

234.29 DISTRIBUTION OF GANGLION CELLS IN THE RETINA OF THE THIRTEEN-LINED GROUND SQUIRREL. A. Flores and E. Kicliter. Lab. of Neurobiology and Dept. of Anatomy, Univ. of Puerto Rico Sch. of Med., San Juan, PR 00901.

The distribution of retinal ganglion cells of the thirteen-lined ground squirrel (Spermophilus tridecemlineatus) was studied in Nissl-stained and horseradish peroxidase-labeled wholemount preparations (Stone, The Wholemount Handbook, 1981; de Olmos and Heimer, Neurosci. Lett. 6: 107-114, 1977). Ganglion cells were measured and counted from camera lucida drawings. Maps of the retinas showing isodensity contours of ganglion cell distribution were prepared. The overall distribution of ganglion cells included a visual streak below the linear optic nerve head and a preponderance of cells in the inferior retina. Ganglion cell densities ranged from 10,000 cells/mm^2 in the visual streak to 2,000 cells/mm^2 in the periphery of the retina.

Frequency/cell size histograms were made for retinas of six animals. The distribution of ganglion cells according to soma size was studied in various regions of the retinas. Ganglion cells ranged in size from 6 to 20 um in diameter. The average ganglion cell soma size increased with eccentricity from the visual streak and large cells were more numerous toward the retinal periphery. Medium-sized (10-14 um diameter) cells were present throughout the retina and together with the small cells were abundant in the visual streak.

Approximately 80% of the cells labeled after injection of horseradish peroxidase into the optic nerve correspond to the cells defined as ganglion cells in the Nissl preparations. The 20% remaining are likely displaced ganglion cells which were not classified as such in the Nissl preparations. These cells were more numerous in the periphery of the retina.

Our findings indicate that the inferior retina, corresponding to the superior visual field, is most critical for vision in this species. Further, as in most mammals, small and medium-sized ganglion cells are abundant in retinal areas associated with acute vision.

Supported, in part, by USPHS Grant NS-07464.

234.30 RETINAL GLIAL CELL ENDFEET HAVE HIGH K^+ CONDUCTANCE. Eric A. Newman . Eye Research Inst. of Retina Fdn., Boston, MA 02114.

Previous investigations have suggested that the endfoot process of retinal Müller cells has high K^+ conductance. I have tested this proposal in two ways, by measuring Müller cell impedance in retinal slices and by monitoring Müller cell depolarization evoked by local extracellular ejection of K^+ in dissociated Müller cells. Recordings were made from cells of the frog, Rana pipiens and the turtle, Pseudemys scripta . Müller cell impedance was measured by passing lnA current pulses through an intracellular electrode and a bridge circuit in a perfused retinal slice preparation. When cells were impaled in their cell bodies (inner nuclear layer) the measured resistance was 8.4 +/- 4.9 MΩ . On the other hand, when cells were penetrated in their endfeet (optic fiber layer), the resistance was 4.9 +/- 3.9 MΩ . This result demonstrates that the Müller cell has a significant cytoplasmic resistance (the cell is not isopotential) and that the conductance of the endfoot region is at least twice the conductance of the cell body region. (The measured conductance ratio between the two regions is less than the actual ratio due to resistive coupling between the regions.)

Local Müller cell membrane K^+ conductance was assessed by recording intracellular responses to local increases in extracellular K^+ concentration ($[K^+]_o$) produced by pressure ejecting a KCl solution from an extracellular pipette. Responses were recorded from the cell bodies of single, enzymatically dissociated cells. $[K^+]_o$ increases near the endfoot region of the cell elicited significantly greater depolarization than did increases near other regions of the cell. Depolarizations evoked by a 10 ms pressure pulse of 117 mM KCl typically had amplitudes of 20 to 30 mV for ejections near the endfoot and 1 to 5 mV for ejections near the stalk, cell body and distal end of the cell. Response latencies and waveforms were similar for K^+ ejections near all cell regions. These results are most easily explained by postulating that the K^+ conductance of the Müller cell endfoot is significantly greater than the conductance of other regions of the cell.

A high endfoot membrane conductance would have an important influence on the magnitude and path followed by currents generated by Müller cell depolarization (as may occur in electroretinogram generation). This membrane specialization might also function as part of a passive glial cell $[K^+]_o$ buffering system; K^+ flowing into Müller cells in regions of increased $[K^+]_o$ would exit through the high conductance endfoot process of these cells, directly into the vitreous. (Supported by NIH grant EY04077.)

234.31 RESPONSE OF RETINAL GANGLION CELLS TO BRIEF LASER EXPOSURES. Randolph D. Glickman, William R. Elliott III*, and David Schafer*, Life Sciences Division, Technology Incorporated, San Antonio, TX.

Brief (<1 msec) flashes of light result in longer dark adaptation times and longer-persisting afterimages than would be predicted from the amount of visual pigment bleached (Rushton, W.A.H. & H.D. Baker, Nature, 200:421, 1963), possibly due to the production of desensitizing photoproducts. Because Q-switched lasers produce light pulses of pico- or nanosecond duration, the effect of these emissions on the retina might be to produce even longer-lasting afterimages. Ganglion cell activity should reflect the presence of afterimages, thereby providing a physiological measure of the degree of flashblindness induced by the laser exposure. In addition the effects of beam size and light scatter within the eye could be evaluated.

Ganglion cell recordings from feline and rhesus monkey retinae were made during exposure of the receptive fields of these cells to laser light. Pulses of light of 530 nm wavelength and 20 nsec duration were obtained from the doubled output of a Q-switched, Nd-glass laser and presented along with white or colored light from a xenon-arc lamp in Maxwellian view to the animal. The intensity of the exposures was adjusted to 0.1 to 1.0 times the Maximum Permissible Exposure (ANSI standard Z136.1, 1980). In the cat retina, a single pulse of laser light, ranging in diameter at the retina from 0.25 to 2.0 deg of visual angle, elicited in ganglion cells a characteristic response: first a brief discharge at the time of exposure, followed by a silent period lasting less than 0.5 sec, and then a maintained after-discharge of spikes at a relatively constant frequency. This after-discharge lasted up to 5 min, although it was briefer in cells more than 10 deg from the area centralis in the cat, possibly due to the Stiles-Crawford effect. During the after-discharge, the threshold for light sensitivity was elevated by up to 4 log units, with recovery completed by the end of the after-discharge.

Similar patterns of response were observed in monkey retinal ganglion cells, except that recovery was faster and was generally complete in about 30 sec. Immediately following the laser exposure, the visual threshold was elevated by up to 2 log units. Recovery of sensitivity occurred shortly before the episode of spontaneous firing ended. These effects were observed even when the laser beam fell 3-5 deg from the receptive field. These single unit responses to laser exposures have similarities to attributes of psychophysical flashblindness, i.e. the persistence of an afterimage and the elevation of the visual threshold.

Supported by Contract F33615-80-C-0610, USAF School of Aerospace Medicine, Brooks AFB, TX.

234.32 PROTECTIVE EFFECT OF TAURINE ON PEROXIDATION REACTIONS IN PHOTORECEPTORS. (SPON A. Feria) Herminia Pasantes-Morales and Carlos Cruz. Centro de Investigaciones en Fisiología Celular, Universidad Nacional Autónoma de México.

Lipid peroxidation has been implicated in the destruction of rod outer segments (ROS) in light-damaged retinas. Experimental models of retinal degeneration produced by intravitreal injection of ferrous sulfate have shown a relationship between lipid peroxidation and alterations in the membranous structure of frog ROS in vivo (Wiegand et al., ARVO Abst. p. 183).

Disturbances produced by constant illumination or by exposure to ferrous sulfate on ROS structure may be reproduced in vitro in isolated frog ROS. In these two experimental models we observed a characteristic alteration of the ordered membranous structure of ROS, accompanied by an increase in malondialdehyde formation. ROS appear swollen, enlarged, showing distention of discs and vesiculation.

The presence of the sulfonic amino acid taurine, in the incubation medium protects against the disturbing effects of light or ferrous sulfate. Taurine protective action is dose-dependent, being maximal at 25 mM. The physiological concentration of taurine inside photoreceptors is about 20-40 mM. For protection of ferrous sulfate-induced damage, zinc should be also present in the incubation medium, although zinc has no protective action by itself. Taurine has no effect on malondialdehyde formation suggesting that its protective effect is not related to a direct action preventing lipid peroxidation. A suggested mechanism for taurine protective action is an effect on ion permeability on ROS membranes. Both, illumination and ferrous sulfate induced damage require sodium chloride and bicarbonate to exert their disturbing action. Therefore, an alteration of membrane permeability allowing ion entry accompanied by water, may be a secondary effect of lipid peroxidation, leading to swelling and structure disruption. Taurine effects on membrane permeability have been described in excitable tissues as well as on isolated ROS. Therefore, taurine protective action may be related to a restoration of membrane permeability altered by peroxidative conditions.

Supported by grant 2 RO1 EY 02540-04 from NIH

234.33 MONENSIN, A CARBOXYLIC IONOPHORE, STIMULATES CALCIUM UPTAKE IN THE RAT RETINA IN THE PRESENCE OF ATP AND ATP PLUS TAURINE. J.B. Lombardini. Department of Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX. 79430.

It has been demonstrated by biochemical and autoradiographic techniques that taurine is the most abundant amino acid in the retina, attaining concentrations as high as 80 mM in the photoreceptor layer (Orr et al., J. Neurochem. 26: 609-611, 1976). However, the precise function of taurine in the retina is unknown, although it has been postulated that taurine has a role as an inhibitory neurotransmitter or as a modulator in the retina. Recent information suggests that taurine has an effect on calcium uptake in excitable tissues. It has been demonstrated that taurine produces an increase in ATP-dependent calcium uptake in retinal preparations either positively or negatively depending upon the calcium ion concentration (Lopez-Colome and Pasantes-Morales, Exp. Eye Res., 32: 771-780, 1981).

Previously it has been reported from our laboratory that at low calcium ion concentration (10 μM) valinomycin, a neutral ionophore, inhibits the taurine-stimulated ATP-dependent calcium ion uptake in rat retinal membrane preparations (Lombardini, Invest. Ophthalmol. Vis. Sci. 24: 161, 1983). The inhibitory effect of valinomycin on calcium ion uptake was observed only when potassium ions were present in the incubation media; this was an expected result since valinomycin is considered to be a potassium ionophore with an equilibrium selectivity for potassium ions over sodium ions of approximately 10,000:1.

Present studies have demonstrated that monensin (MNS), a carboxylic ionophore which has an equilibrium selectivity ratio of 10:1 for sodium ions over potassium ions, stimulated the uptake of calcium ions by rat retinal membrane preparations. Replacement of the sodium salts in the buffer with potassium salts did not lessen the stimulatory effects of monensin.

Condition	Control	ATP (1.2 mM)	ATP (1.2 mM) + Taurine (20 mM)
Na^+ buffer	0.10 ± 0.02	0.50 ± 0.14	2.03 ± 0.36
Na^+ buffer + MNS	0.21 ± 0.08	1.47 ± 0.26[a]	3.16 ± 0.35[a]
K^+ buffer	0.23 ± 0.08	0.91 ± 0.11	1.85 ± 0.14
K^+ buffer + MNS	0.35 ± 0.11	1.93 ± 0.45[a]	4.09 ± 0.80[a]

Na^+ buffer = bicarbonate buffer containing sodium salts.
K^+ buffer = bicarbonate buffer containing potassium salts.
N = 4; [a] $P < 0.05$, Duncan's multiple-range test.

(Supported in part by NIH grant EY04780 and by a Biomedical Research Support Grant from Texas Tech University Health Sciences Center.)

234.34 EFFECTS OF GUANIDINOETHYL SULFONATE (GES), A TAURINE DEPLETOR IN THE ADULT RAT RETINA. Norma Lake and Sue Cocker*, Departments of Physiology and Opthalmology, McGill University, Montréal, Canada.

The rat retina contains high levels of the sulfur-containing amino acid, taurine, which may be rapidly depleted by in vivo treatment with a taurine analogue, GES, (Lake, Life Sci. 1981, 29: 445). The present experiments indicate that GES is an antagonist of high affinity taurine uptake in the isolated retina in vitro. Taurine concentration was 10 μM, and a range of GES concentrations gave the following inhibitions of uptake:

GES conc'n (μM)	Uptake (%control)	n	t-test
125	94	16	n.s.
500	61	16	p<.001
1000	49	16	p<.001

Extracellular space and binding corrections were estimated using labelled mannitol or 3°C incubations. In addition, the presence of GES did not accelerate the efflux of previously accumulated taurine. The biosynthetic capacity for taurine of the retina is low as estimated from enzymic activities. Thus it would appear that the taurine-depleting effects of GES observed in vivo arise mainly due to antagonism of taurine transport across the blood-retinal barrier.

Electroretinographic studies (ERG's) were carried out on male rats (Sprague Dawley, 260g at start) who were recorded for several weeks to establish control values before being placed on the GES treatment (1% in drinking water). Analyses of averaged potentials to full field white or coloured flash stimuli over a range of intensities were made to determine a and b wave latencies, implicit times and amplitudes, and the relationship between these quantities and stimulus intensity. Treatment with GES led to significant changes in 4 weeks: b wave amplitudes decreased by 21-42% and the slope of b wave amplitude versus intensity decreased by 31-52% compared to control. These effects were partially reversible (amplitudes recovered to 85-96% of control) by 3 weeks after discontinuing GES treatment. These studies indicate the functional importance of taurine in retinal activity.

Supported by the Medical Research Council of Canada.

234.35 **EFFECTS OF SOME NEUROTRANSMITTER CANDIDATES ON THE RESPONSES OF SKATE (RAJA ERINACEA) GANGLION CELLS.** **J. Cohen. Dept. of Ophthalmology, New York Univ. Med. Ctr., New York, 10016.**

Immunocytochemical studies (Brunken, Karten and Witkovsky, 1983) have demonstrated the presence of the neurotransmitters GABA and serotonin in the retina of the skate Raja erinacea. In addition we will present autoradiographic evidence for the presence of glycine-accumulating cells. We therefore wanted to determine the physiological action of these transmitter candidates on the responses of ganglion cells in the retina of the skate.

Extracellular recordings were made from the dark-adapted, superfused eyecup preparation using platinum-iridum electrodes. The majority of cells recorded from was of the ON-center variety. The action of glycine, in doses as low as 500 μm, was to inhibit the light-evoked responses. This action was blocked by addition of 100 μm strychnine to the glycine-containing superfusate. Those cells that were inhibited by glycine were not affected by GABA. However, GABA, in doses as low as 500 μm inhibited the on discharge of other ON-center ganglion cells which in turn were not affected by glycine. Area-sensitivity curves were determined for all these cells by finding a threshold response to spots of increasing diameter. The resulting curves could be described by a power function with slopes ranging from 1.43 to 1.82. The integrating area for the cells inhibited by glycine was smaller than for those affected by GABA. Preliminary data suggest that serotonin had an excitatory action on ganglion cell responses.

Supported by Grant EY07009.

234.36 CONTROL OF DARK-INDUCED RELEASE OF ^{3}H-GABA FROM GOLDFISH RETINAL HORIZONTAL CELLS. S. Yazulla and K. Studholme*. Dept. of Neurobiology and Behavior, S.U.N.Y. Stony Brook, NY 11794.

H1 horizontal cells of goldfish retina probably are GABAergic. They receive synaptic inputs from red cone photoreceptors which may use glutamate or aspartate as their transmitter, and from dopaminergic interplexiform cells (DA-IPC). H1 cells are synaptically excited by red cones in the dark and should release GABA in this condition. DA-IPCs appear to be inhibitory since the application of dopamine reduces the receptive field size of H1 cells. D-aspartate has been found to potentiate the depolarizing action of L-glutamate on H1 cells, presumably by blocking the uptake of L-glutamate. If D-aspartate were to block the uptake of the red cone transmitter, then H1 cell excitation should be enhanced resulting in increased and hopefully detectable ^{3}H-GABA release. This study was designed to detect release of ^{3}H-GABA from H1 cells by a physiological stimulus (darkness) in order to obtain information regarding the identity of the red cone transmitter and on the action of DA-IPCs on the synaptic release of GABA by H1 cells.

The release of ^{3}H-GABA was studied by biochemical analysis of perfused isolated retinas. Retinas were isolated from goldfish which were light adapted in very dim room light for 2 hrs. Retinas were incubated in dim red light for 20 min in 0.72 μM ^{3}H-GABA, placed in a perfusion chamber (flow rate-1 ml/min), rinsed for 30 min in dim red light and subjected to darkness under a variety of conditions. Radioactivity in the perfusate was determined by liquid scintillation spectroscopy. The findings are: 1. both L-glutamate and L-aspartate cause a dose-dependent release of ^{3}H-GABA from H1 cells which is independent of extracellular calcium, 2. inclusion of 3.2 mM D-aspartate in the perfusion medium potentiates the effect of L-glutamate and totally inhibits L-aspartate, 3. retinas perfused in the standard Ringer without D-aspartate do not show increased ^{3}H-GABA efflux when placed in the dark, 4. when 3.2 mM D-aspartate is included in the perfusion medium, there is significant dark-induced ^{3}H-GABA release at a rate of 0.65 nmoles [3-H] GABA/retina/min. This is only about 10% of the release rate induced by 1 mM L-glutamate, 5. 100 μM dopamine reversibly inhibits the dark-induced release of ^{3}H-GABA. These results show that release of ^{3}H-GABA from H1 cells can be detected under physiological conditions strongly supporting the hypothesis that H1 cells are GABAergic and, in addition, are subject to antagonistic inputs from red cones and DA-IPCs. Furthermore, since D-aspartate potentiates ^{3}H-GABA release evoked by L-glutamate and inhibits L-aspartate, and, is required for the detection of dark-induced release of ^{3}H-GABA, the transmitter for red cones more likely is L-glutamate rather than L-aspartate.

Research supported by NIH grant EY01682 to S.Y.

234.37 KAINIC ACID STIMULATES THE RELEASE OF ENDOGENOUS AMINO ACIDS FROM CHICK RETINA IN VITRO. P. Campochiaro*, J.W. Ferkany and J.T. Coyle. Depts. of Opthalmology, Psychiatry, Neuroscience, Pharmacology, Div. of Child Psychiatry. Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

The spontaneous and evoked release of amino acids from isolated chick retinas was studied by means of a sensitive precolumn derivitization HPLC technique. Retinas were dissected from chick eyes, placed in perfusion chambers, and superfused at 0.5 ml/min with Krebs bicarbonate buffer bubbled with O_2:CO_2(95:5) and maintained at 37°C. After one hour perfusion, spontaneous release of taurine (Tau), glycine (Gly) and glutamine (Gln) were found to be > ten-fold greater than that of aspartate (Asp), glutamate (Glu) and GABA. Increasing concentrations of KCl substituted for NaCl caused a dose-dependent release of all six amino acids although maximal response for Gln was only 23±4% (N=6;p<0.05). The EC_{50} values for release of Tau (+235±25%), Gly (+70±8%) and Glu (+50±8%) were approximately 30 mM KCl whereas the EC_{50} for GABA release (+215±40%) was 50 mM KCl. Notably, whereas the KCl-evoked release of Glu, Gly and GABA peaked within 4 min, the KCl-release of Tau was gradual and peaked 8-12 min after stimulus onset. Ca^{++}-free buffer containing 2mM EGTA inhibited 30 mM KCl evoked release of Glu, GABA and Gly by >90% whereas Tau release was inhibited by only 55±5% and Asp by 67±7%.

Kainic acid (KA;100 uM) was quite effective in evoking release of Tau (+477±130%), Gly (624±58%) and GABA (1258±240%) but not Gln, Asp or Glu in contrast to the effects of KA on perfused brain slices (Ferkany et al, Nature 298:757,1982). The EC_{50} of KA was 30 uM for Gly, 36 uM for GABA and 50 uM for Tau. Intravitreal injection of 125 nmoles of KA results in a severe loss of cells in the inner nuclear layer but spares the outer nuclear layer and the ganglion cells. This lesion reduced the receptor specific binding of [^{3}H]-KA(25nM) to retinal homogenates to 29±4% of control (N=6;p<0.01). The KA lesion markedly decreased 100 uM KA evoked release of Gly (-86±1%), Tau (-99±4%) and GABA (-97±1%). The 30 mM KCl evoked release from KA lesioned retina was also markedly reduced for Gly (-85±4%) and GABA (-97±3%); however, the release of Tau (120±10%) and Glu (67±15%) did not differ significantly from that of intact retinas.

The results are consistent with a direct, receptor mediated release of Gly and GABA by KA; in contrast, the effects of KA on release of Tau appear to be indirect and may involve a different pool than that evoked by 30 mM KCl. (Supported by a fellowship from Fight for Sight of New York and USPHS grant NS 13584).

235.1 MORPHOLOGICAL DIFFERENCES IN RETINAL GANGLION CELLS PROJECTING TO DIFFERENT LAYERS OF THE DORSAL LATERAL GENICULATE NUCLEUS IN THE TREE SHREW. E. J. DeBruyn* and V. A. Casagrande (SPON: J. K. Brunso-Bechtold). Depts. of Anatomy and Psychology, Vanderbilt Univ., Nashville, TN 37232.

Previous studies of the dorsal lateral geniculate nucleus (LGN) of the tree shrew have shown that this nucleus contains 6 well defined layers, two of which receive uncrossed retinal input (1,5) and four of which receive crossed input (2,3,4 and 6). These layers can also be distinguished on the basis of cytology, cortical projections and physiology. Layers 1 and 2 have medium and large spindle shaped cells that project to the upper tier of layer IV of striate cortex; layers 4 and 5 contain medium and large sized darkly staining cells that project to the lower tier of layer IV; and layers 3 and 6 contain smaller, pale staining cells that project to the supragranular cortical layers. Physiological studies have shown that layers 1 and 2 contain ON center cells, layers 4 and 5 contain OFF-center cells and layers 3 and 6 contain OFF-center (layer 3) or ON-OFF-center (layer 6) cells.

In light of the above, we were interested to see if retinal ganglion cells (RGCs) projecting to different layers of the LGN formed anatomically distinct populations. Iontophoretic injections of HRP were made into single or multiple laminae of the LGN in 10 tree shrews using evoked potentials to determine laminar boundaries. Following a two day survival, the animals were perfused and the retinae were processed with Hanker-Yates reagent, whole mounted and stained with cresyl violet. The areas of labeled and unlabeled RGC somas were measured at 1000X and the level of dendritic ramification within the inner plexiform layer (IPL) was noted for well-filled cells. Results show the following: RGCs projecting to layer 3 have small (1st-35th %tile of local cell sizes) somas, while those projecting to layer 6 are primarily small with a few medium (36th-70th %tile) and large (71st-99th %tile) cells also. RGCs projecting to layers 1,2,4 and 5 are medium and large sized and can be further distinguished in terms of the level of dendritic ramification within the IPL. Those projecting to layers 1 and 2 ramify in the superficial IPL and those projecting to layers 4 and 5 ramify in the deep part of this layer. Taken together with earlier studies, these results suggest that the RGCs projecting to different LGN laminae are morphologica ly distinct and that the information they carry ends in different cortical sublaminae Further, it suggests that ON and OFF center information reaches cortex via separate channels in this species Support by EY01778; 1K04-EY00223; MH 08472; RR 05424

235.2 PROJECTIONS OF THE INTERNAL OPTIC TRACT: RETINAL PROJECTIONS WHICH SURVIVE SUPERFICIAL THALAMIC LESIONS IN HAMSTERS. G.E.Schneider and S.Jhaveri* (SPON: J.Delatizky). Department of Psychology & Research Lab. of Electronics, Massachusetts Institute of Technology, Cambridge, MA 02139.

Retinal ganglion cell axons cross at the chiasm and form a broad ribbon as they traverse the external surface of the cerebral peduncle. Many of these axons remain on the thalamic surface as they course over the lateral geniculate body (LGB) and continue caudally to the superior colliculus (SC). Collaterals from these surface axons dive into the LGB and terminate along characteristic lines of projection. However, a fair number of retinofugal axons leave the surface optic tract and penetrate the ventral nucleus of the LGB, immediately dorsal to the peduncle. These axons, running parallel to the superficial fibers, travel in tight fascicles through the LGB, the lateral-posterior nucleus (LP) and the pretectum forming what we will refer to as the internal optic tract (IOT).

To study the projections of the IOT in adult Syrian hamsters, the dorsal thalamus was exposed and shallow knife cuts were made across the optic tract, confined in some cases to the outer 1/5 of the LGB. After survival periods of up to 4 weeks, the contralateral eye was injected with a solution of WGA-HRP and/or ^{3}H-amino acids. Animals were sacrificed after a further survival of 24-36 hours and the brains treated according to the TMB procedure for visualization of the transported enzyme; appropriate cases were prepared for standard autoradiography.

IOT axons can be traced through the deeper portions of LGB and LP, with little evidence of dense arborization until they reach the pretectal olivary nucleus, where they show heavy termination even when only the deepest IOT axons are spared by the initial lesion. The nucleus of the optic tract is, at best, only sparsely innervated by the IOT, whereas the posterior pretectal nucleus receives axons from its more superficial portions. In the rostral SC, the IOT does not contribute to the dense fascicles of the stratum opticum; it appears to retain its deeper-lying position. Caudally this separation becomes obscured. In the superficial gray layer (SGS), the deepest axons of the IOT appear not to terminate near the tectal borders, in the representation of the peripheral retina. Even when most of the IOT is spared, the central portions of the SGS are more densely labelled than the margins The IOT axons also appear to terminate in the intermediate gray layer along their trajectories.

These findings have implications for studies of optic tract development and plasticity, as well as for studies of visual system function.

Support: NIH grants 5R01-EY00126 & 5P30-EY02621 & Ortho Labs

235.3 HUMAN PATTERN EVOKED RETINAL CORTICAL AND RESPONSE: EFFECTS OF SPATIAL AND TEMPORAL ADAPTATION, J. Vernon Odom. Department of Ophthalmology, West Virginia University, Morgantown, WV 26506.

If the optic nerves of humans and cats are severed unilaterally and the eye on the ipsilaterial side is stimulated with pattern reversal or luminance increments, the response to luminance remains normal while the response to pattern is extinguished suggesting that retinal potentials elicited by pattern reversal stimulation originate in retinal ganglion cells (Maffei and Fiorentini, 1981; Dawson et al, 1982). Use of pattern evoked retinal responses in humans appears then to represent a means of examining the population behavior of human retinal ganglion cells Comparisons of retinal and cortical response may aid our understanding the retinal role in determining cortical responses.

In three experiments the effects of adaptation on retinal and cortical responses were examined. Sinusoidal gratings of 30% contrast were alternated at 4 Hz (8 alt/sec). In the first experiment subjects were adapted for two minutes either to an unpatterned field of the same mean luminance as the test field or a pattern of the same spatial (1 cpd) and temporal frequency (4 Hz; 8 alt/sec). In a second experiment test patterns of 30% were alternated at 4 or 16 Hz (8 or 16 alt/sec) following 2 min. adaptation to 50% contrast patterns of the same temporal but varying spatial frequencies. The bandwidth of spatial frequency channels may be defined by the half-amplitude points of the adaptation induced amplitude reduction. In the third experiment patterns of 30% contrast alternated at 4 and 16 Hz were presented following two minutes of adaptation to whole field flicker and to patterns of the same spatial frequency and 50% contrast alternated at varying temporal frequencies, providing an indication of the bandwidth of temporal channels.

235.4 RETINAL EFFERENTS AND AFFERENTS IN A CICHLID FISH. A.D. Springer and A.S. Mednick. Department of Anatomy, New York Medical College, Valhalla, NY 10595.

Cobaltous-lysine was applied to the severed optic nerve of oscars (Astronatus ocellatus) and they were allowed to survive for 24 hr at 18°C. Thereafter, the cobalt was precipitated and paraffin sections were intensified with silver. Retinal fibers terminate bilaterally in a ventral preoptic region between the base of the third ventricle and the genu of the horizontal commissure and among periventricular cells along the sides of the third ventricle. Other fibers innervate the tuberal region of the hypothalamus anterior to the infundibulum. Targets innervated in the pretectum include: nucleus lateralis geniculatus and dorsal, medial and ventral pretectal nuclei. Two targets in the dorsal (nucleus opticus dorsolateralis and nucleus opticus commissurae posterior) and one in the ventral thalamus (nucleus opticus ventrolateralis) are innervated by fibers that leave the medial edge of the dorsal optic tract, while two targets in the ventral thalamus (basal optic nucleus and accessory optic nucleus) are innervated by fibers from the ventral optic tract.

Retinal fibers in the ventral optic tract innervate the ventral tectum, but not rostrally. Fibers in the dorsal optic tract innervate the entire rostro-caudal extent of the dorsal tectum and, in addition, the rostral pole of the ventral tectum. Thus, the axons of retinal ganglion cells from dorsal and ventral hemiretinas are not completely segregated in the optic tracts since the axons of dorsotemporal retinal ganglion cells are located in the dorsal optic tract.

Efferents to the retina originate in two nuclei and both project contralaterally. The first is the nucleus olfactoretinalis which is located ventrally between the olfactory lobe and forebrain, and consists of about 200 cells. Most of the cells are 11-13 um in diameter, with a few cells having a 20 um diameter The second, nucleus thalamoretinalis, is an arched and diffuse system of 200 cells extending from anterodorsal to posteroventral thalamus. Most of the cells are 8-10 um in diameter, but a few cells are 20 um in diameter. Cobalt-filled cells were not observed in either tectal lobe.

Supported by EY-03552.

235.5 EXCITATORY AND INHIBITORY RECEPTIVE FIELD ORGANIZATIONS OF RETINAL GANGLION CELLS IN TURTLE. J.E. Fulbrook**, J.H. Maxwell, and A.M. Granda. Inst. for Neuroscience, Univ. of Delaware, Newark, DE. **U.S. Army Aeromedical Research Lab., Ft. Rucker, AL.

Excitatory (ERF) and inhibitory (IRF) receptive fields (RFs) were investigated with single-unit, extracellular recordings taken from retinal ganglion cell (RGC) axons responding to moving and/or flashing light spots in the curarized, anesthetized turtle, Pseudemys. The RF organization of most RGCs was complex, usually asymmetric, and frequently mutable to changes in adaptation level, stimulus wavelength, and stimulus velocity. All of the units were particularly sensitive to moving stimuli. 40% of the units were directionally-sensitive (DS).

In turtle, most RFs have ERF and IRF regions but IRF regions are usually silent, not giving well-defined, robust responses to moving or flashed stimuli. Also, the spontaneous activity in these units was very low (less than one spike/sec) or even non-existent, especially when light-adapted. IRF regions in these units could still be studied by mapping RFs with a moving stimulus while a supplementary flashing stimulus was used to drive the cells. This technique only works with slow-adapting cells; however, from these units several complex ERF-IRF organizations were revealed. DS cells showed ERF and IRF regions in the preferred direction of stimulus movement, but had only an IRF region in the null direction, overlapping the ERF-IRF region seen in the preferred-direction RF map. At least four ERF-IRF organizations were described for DS and bar-shaped (orientation-sensitive) cells.

Directionally-preferring (DP) cells were found that differed from DS cells in that DP cells had their strongest ERF and IRF regions in the preferred direction but had weak ERF-IRF regions in the null direction. In effect, DP cells had opposite IRF-strength vectors from DS cells, and possibly represent a separate cell class.

Rest assured, not all turtle RGC RFs are complex and unusual. Some units were studied which had relatively simple center-surround ERF-IRF organizations and at least one of these will be shown.

Summary: In the number and variety of complex RF types described at the RGC level, the turtle possesses one of the most complex retinal integrative capacities recognized in vertebrates.

Supported by N.E.I. grant #01540.

235.6 TIME DEPENDENCE OF ACTIVITY IN FROG RETINAL GANGLION CELLS, M. Stiles,* K. P. Unnikrishnan* and E. Harth. Physics Dept., Syracuse Univ., Syracuse, N.Y. 13210.

Neural activity was recorded from neighboring retinal ganglion cells in frog (Rana pipiens) optic tectum, using extracellular tungsten microelectrodes. Action potentials were labeled on-line according to arrival time and waveform, and stored on flexible disk. Photic stimulation by cathode ray tube consisted of rectangles of chosen size, contrast and position within the previously determined receptive field. The post-stimulus time histogram of the accumulated responses showed a high degree of reproducibility. About 70% of the recordings show bursts lasting several hundred milliseconds, often with a marked periodic pattern.

Inter-burst intervals change regularly with stimulus contrast, size and position. In some cases, delayed burst responses appear several hundred milliseconds following a very brief (17 msec.) stimulus.

We have investigated the interactions between the temporal patterns produced by temporally or spatially separated stimuli on the supposition that temporal coding of ongoing activity describes complex visual events.

(Supported in part by BRSG Grant SO7 RR077068-17, NIH)

235.7 A DOSE-RESPONSE ANALYSIS OF THE EFFECTS OF ANTIBODIES TO LARGE GANGLION CELLS ON THE CAT'S RETINOGENICULATE PATHWAYS. John W. Crabtree*, Peter D. Spear, Lillian Tong, Kim R. Jones and Steven E. Kornguth. Depts. of Psychology and Neurology, Univ. of Wisconsin, Madison, WI 53706.

Antibodies were produced against large ganglion cells derived from ox retinae. These immunoglobulins were injected into the vitreal chamber of one or both eyes in adult cats. Antibody concentration ranged from 333-1000 ug/0.1 cc injection volume and the animals were studied after at least a 3 week postinjection period.

Evoked potentials to optic chiasm shock were recorded from the optic nerve head of 22 eyes. With increasing antibody concentration the ratios of the Y- to X-wave amplitudes decreased monotonically from 0.71 to 0.05. Relative to evoked potentials obtained from noninjected and control eyes, those from injected eyes showed a substantial reduction in both X- and Y-wave absolute amplitudes for all concentrations used. However, with increasing concentration the amplitude of the X-wave remained constant while that of the Y-wave steadily declined. In fact, 3 of 6 eyes injected with the 1000 ug concentration showed an absence of the Y-wave.

In 8 cats monocularly injected (1000 ug concentration), single cells were recorded from either the retinal ganglion soma layer or laminae A and A1 of lateral geniculate nucleus (LGN). While the injected-eye sampling density for retinal W-cells (13.8%) was unaffected, the densities for X- and Y-cells (8.6% and 1.5% respectively) were significantly lower than the noninjected-eye densities (17.3% and 12.7% respectively). These effects are in contrast to the lack of retinal effects reported for cats monocularly injected with 333 ug antibody concentration (Spear et al., 1982).

From some of the above cats retinal wholemounts were prepared and stained with cresyl violet. Thus far, cell counts have revealed an average of 93.5% fewer alpha cells (presumed Y-cells) in the injected eye than in the noninjected fellow eye. This average is a 3-fold greater loss of alpha cells than the average following injections of 333 ug concentration (Kornguth et al., 1982).

In LGN laminae receiving input from the injected eye, only 2% of the encountered cells (1/50) were Y-cells; the proportion of encountered X-cells (61.6%) was normal. The proportion of Y-cells was significantly lower than the 29.6% Y-cells (14/47) encountered in LGN laminae receiving input from the noninjected eye. This difference represents a 93% loss of Y-cells in injected-eye laminae, which is greater than the 77% Y-cell loss in such laminae in cats that received concentrations of 333 ug (Spear et al., 1982).

These findings show that the effects of antibodies on retinofugal pathways in the cat are dose-dependent. Furthermore, by using a sufficiently high antibody concentration, a nearly complete loss of the Y-cell pathway is possible with only a partial loss of X-cell and no loss of W-cell pathways. (EY01916, EY02545)

235.8 SPECIALIZATIONS IN THE VISUAL SYSTEM OF PANTODON BUCHHOLZI (Teleostei, Osteoglossiformes). William M. Saidel and Mark R. Braford, Jr., Dept. of Anatomy, Georgetown University Medical School, Washington, D.C. 20007

The visual system of P. buchholzi exhibits several atypical features. The retina is divided along a horizontal axis into two parts by an elaborated falciform process. We show that the ventral hemiretina views through the water-air interface while the dorsal part of the retina views the aquatic environment. The two parts of this retina are different in various aspects of the neuronal architecture. The ventral hemiretina is more complex in all features that were compared.

We have investigated the central connections of the retina as a whole and of the dorsal and ventral hemiretinas individually using autoradiographic, degeneration, and HRP histochemical techniques. The dorsal and ventral hemiretinas each contribute a separate fascicle to the optic nerve (ON). The two fascicles fuse outside the sclera into the ON. At the chiasm, an ON almost completely decussates, with most fibers entering the contralateral optic tract (OT). The OT continues along the lateral margin of the diencephalon to form the Marginal OT (MaOT). Two fascicles leave the MaOT to innervate the Superchiasmatic Nucleus. Further caudal, the MaOT divides into medial (MOT), lateral (LOT), and dorsal (DOT) optic tracts. The MOT terminates in several midline nuclei including the Anterior, Intermediate, and Ventrolateral Thalamic nuclei. The tract continues into a periventricular pretectal nucleus (PP), with fibers terminating and others crossing to the ipsilateral PP. Some of the crossed fibers continue rostrally within the ipsilateral MOT. At the rostral pole of the optic tectum (OpT), fibers from the LOT terminate in the Superficial Pretectal nucleus, pars parvocellularis, in a pretectal complex, and in an accessory optic complex. Fibers from the DOT also innervate the pretectal complex, but the bulk of fibers from both the DOT and LOT terminate in the OpT, primarily deep to the marginal layer and adjacent to the Periventricular Cell Layer (PV). The PV layer exhibits a discontinuity about midway between the medial and lateral margins of the OpT. By tracing the fibers from the ventral or dorsal hemiretina alone, we determined that this discontinuity marks the boundary between terminations from the two hemiretinas. Optic tract fibers that do not cross in the chiasm, travel in the LOT, and terminate only in the lateral OpT, interdigitating with fibers from the dorsal hemiretina only.

This visual system thus exhibits peripheral and central structural differences that parallel differences in visual environments. (Supported by a BRSG grant from GU, and grants from NSF and NIH.)

235.9 THE EFFECTS OF AMBIENT ILLUMINATION ON CONTRAST SENSITIVITY AND DYNAMICS OF THE CAT RETINA AND LGN. R.M.Shapley*, E.Kaplan* and D. Tranchina*† (SPON: F.Ratliff). Laboratory of Biophysics, Rockefeller University, New York, N.Y. 10021 and †Courant Institute, New York, N.Y. 10012.

Regulation of visual contrast sensitivity is one of the main functions of the retina. We have studied this process by recording from optic tract fibers and LGN cells in lightly anesthetized cats and by stimulating the retina with modulated patterns of light over a wide range of mean retinal illumination. The contrast sensitivity (impulse/sec per unit contrast) of retinal ganglion cells and LGN cells increased greatly from the lowest light level we used, approximately 10^3 quanta/(deg^2 sec), up to a mean illumination of 10^8 q/(deg^2 sec). These levels of illumination extend from the low scotopic to the mesopic range of vision for the cat. In several but not all cells, the contrast sensitivity actually declined between 10^8 and 10^9 q/(deg^2 sec) mean retinal illumination (corresponding to luminances of between 10 and 100 cd/m^2 respectively). This drop in contrast sensitivity may be associated with the shift from scotopic to photopic visual function in cat retinal ganglion and LGN cells.

The effect of mean light level in the dynamics of response was determined by measuring the temporal frequency response of each cell over the same range of backgrounds as above, with a small centrally placed spot modulated sinusoidally in time around the mean level. There was a differential effect of mean level on responses at low temporal frequencies versus high temporal frequencies. At low temporal frequencies the gain (impulses/quantum) was approximately 1/(mean level), while at high temporal frequencies the gain was approximately constant, i.e. unaffected by mean level. The phases of the responses were affected in a consistent fashion: phases of the responses to low frequencies were advanced by increasing mean level, while phases of the responses to high frequencies were relatively unaffected by variations in the mean level of illumination. These results suggest that the retinal processes which control gain and dynamics modulate the strength of negative feedback rather than causing a multiplicative attenuation of retinal responses to light.

This work was supported by NIH grants EY01472, EY00188, and EY01428.

235.10 MORPHOLOGY OF NORTH AMERICAN OPOSSUM RETINAL GANGLION CELLS. P. D. Wilson and G. J. Condo, Dept. of Psychology, Univ. of California, Riverside, Riverside, CA 92521.

Previous studies of retinal ganglion cells (RGC) in the North American opossum (Didelphis virginana) have suggested three or possibly four classes of RGC based on soma size distributions (Rapaport, et al., JCN, 199: 465, 1981), conduction velocity groups (Rowe, et al., JCN, 199: 481, 1981), and pattern of central projection (Rapaport and Wilson, JCN, 213: 74, 1983). In order to further investigate the anatomical characteristics of opossum RGC, we have examined in retinal wholemounts the dendritic morphology of RGC filled with HRP from injections in either the optic nerve or tract. Retinae were dissected and reacted with either cobalt or cobalt-nickel intensified DAB, and counterstained with cresyl violet. Four distinct types of RGC dendritic morphology were identified. Type 1 RGC are characterized by large dendritic fields (200-375μm) with radiate organization and a relatively complex branching pattern steming from 3-5 primary dendrites (soma diameter:21-32μm). Type 1 RGC in the opossum are similar in dendritic morphology to RGC termed Type 1 in the rat, Class I in the rabbit, and alpha cells in the cat. Type 2 RGC in the opossum are characterized by small to medium dendritic fields (64-202μm), a complex pattern of dendritic branching issuing from 1-2 primary dendrites, and dendritic arborizations displaced from the cell body (17-28μm). These Type 2 RGC are similar in morphology to beta RGC in the cat. Type 3 RGC are characterized by medium size dendritic fields (131-252μm) with bipolar dendritic arborizations from 2-4 opposing primary dendrites and a sparse branching pattern (soma size: 10-27μm). Type 4 RGC are characterized by medium size dendritic fields (134-265μm) with sparse dendritic branching arising from 2-4 primary dendrites..The dendritic branching of Type 4 RGC is in a radiate pattern in contrast to the bipolar pattern of Type 3 RGC. Type 4 RGC are morphologically similar to gamma-epsilon RGC in the cat retina. The present material indicates that there is a significant overlap in soma diameter for morphological classes of RGC in the opossum. It is of interest that for none of the proposed classes was there a systematic change in dendritic morphology with increasing eccentricity from the area centralis. (Supported by NSF grant BNS-8021602 to PDW).

235.11 MORPHOLOGY OF DORSAL LATERAL GENICULATE NUCLEUS RELAY CELLS OF THE NORTH AMERICAN OPOSSUM. G. J. Condo and P. D. Wilson, Dept. of Psychology, Univ. of California, Riverside, Riverside, CA 92521.

The dorsal lateral geniculate nucleus (dLGN) of the North American opossum (Didelphis virginana) is not cytoarchitecturally laminated, but termination of retinal projections show some degree of afferent lamination within the dLGN. The cells in the opossum dLGN (formalin fixed tissue) have a unimodal soma size distribution (11-19μm, mode: 15μm) with little variation through the nucleus. We have investigated the morphological differentiation of dLGN relay cells in the opossum using Golgi-Cox impregnated material and cells filled with HRP retrogradely from visual cortical areas. Both Golgi-Cox and HRP materials was serially sectioned at 120 and 100μm coronally; HRP sections were reacted with either cobalt or cobalt-nickel intensified DAB, and counterstained with cresyl violet. Three cell types have been identified on the basis of soma shape, dendritic morphology, and the orientation of the dendritic arborization. Type 1 relay cells are characterized by round somata (15-18μm in diameter with HRP and 12-26μm in diameter with Golgi-Cox), radial dendritic arborizations issuing from 4-6 primary dendrites, and a dendritic field mean diameter of 200-398μm. Type 2 relay cells have relatively round somata (13-19μm in diameter with HRP, and 13-22μm in diameter with Golgi-Cox), 3-5 primary dendrites giving rise to a vertically elongated dendritic arbor (150-400μm in mean diameter) oriented dorso-ventrally within the nucleus. Type 3 relay cells have relatively fusiform somata (14-16μm in diameter with HRP, and 19-22μm in diameter with Golgi-Cox) 3-5 primary dendrites giving rise to a horizontally elongated dendritic arbor (150-430μm in mean diameter) oriented medio-laterally within the nucleus. All three morphological types of relay cells have been identified throughout the anterior-posterior extent of the dLGN. Thus while there is a unimodal distribution of cells in the opossum dLGN there are three distinct morphological types of relay cells, and there are morphological similarities between Type 1 and Type 2 relay cells in the opossum and Class 1 and Class 2 relay cells in the cat dLGN. (Supported by NSF grant BNS8021602 to PDW).

235.12 AN ELECTRON MICROSCOPIC STUDY OF SYNAPTIC TERMINAL MORPHOLOGY IN THE CHICKEN VENTRAL LATERAL GENICULATE NUCLEUS. R.M. KRAEMER* AND W.J. CROSSLAND (SPON: R. Pourcho). Dept. of Anatomy, Wayne State Univ. School Medicine, Detroit, MI 48201

The neuropil lamina of the chicken ventral lateral geniculate nucleus (GLv) receives projections contralaterally from the retina and ipsilaterally from the visual wulst and optic tectum. We have examined this lamina using the electron microscope to determine qualitatively the morphological categories of synaptic terminals present in order to relate them subsequently to the three sources of afferents.

Four major types of synaptic terminals have been found. (I) An irregularly shaped terminal containing large round synaptic vesicles in a matrix of medium electron density. These terminals contain pale staining mitochondria with poorly defined cristae and a relatively large space between the inner and outer mitochondrial membranes. In newly hatched chicks, the terminals contain inclusions resembling beta glycogen. The terminals form asymmetric synapses on small dendrites, spine-like profiles, and small vesicle containing profiles (VCPs) but rarely contact medium or large dendrites or cell bodies. This terminal usually occurs in a cluster of small profiles resembling the "glomerular" synaptic arrays seen in the cat and monkey thalamus, although it lacks a clearly defined glial wrapping. (II) A rounded terminal containing mainly round (a few pleomorphic) vesicles (smaller in diameter than the vesicles in type I terminals) in an electron lucent background matrix. The terminal makes asymmetric synapses, most frequently on dendritic shafts, less commonly on small dendrites and spine-like processes. Type II terminals frequently are covered on three sides by a glial wrapping. (III) A terminal similar in shape to type II but containing predominantly pleomorphic vesicles in an electron lucent matrix and making symmetrical synaptic contacts with dendritic shafts and small dendritic processes. (IV) VCPs have a few scattered pleomorphic vesicles in an electron lucent matrix and contain medium staining mitochondria with poorly defined cristae. VCPs have not been seen forming presynaptic relationships with other profiles but are commonly post-synaptic to type I and III terminals.

Following eye removal, type I terminals degenerate. We conclude that the chicken has retinal synaptic terminals resembling those found in the visual pathways of other types of vertebrates including the cat and monkey.

(Supported by grant EY-01796 from the National Eye Institute.)

235.13 LIGHT ADAPTATION IN THE TURTLE RETINA AND THE ROLE OF NEGATIVE FEEDBACK. D.Tranchina*[1,2], J. Gordon[1] and R.M.Shapley*[1]. Laboratory of Biophysics, Rockefeller University, New York, N.Y. 10021 and [2]Courant Institute of Mathematical Sciences, New York, N.Y. 10012.

We measured temporal frequency transfer functions of horizontal cell responses to modulations of retinal illuminance superimposed on various steady background levels. These levels spanned the range of $2 \cdot 10^{9}$ - $2 \cdot 10^{12}$ effective quanta sec^{-1} cm^{-2} at 630 nm on the retina. The time to peak of the impulse response to a superimposed flash decreased from 211 msec at the dimmest background to 74 msec at the brightest background. The incremental sensitivity of the response to a low frequency of modulation decreased as the background illuminance increased. Over a range of high background levels, the amplitude of the response to a low frequency stimulus depended only on its contrast and was independent of background. On the other hand, the incremental sensitivity of the response to a high frequency of modulation was independent of background; the amplitude of a high-frequency response was proportional to the absolute amplitude of light modulation, and the phase of the response was also independent of background. Consequently, as the background level is increased, the gain (mV/photon) of the response at high frequencies approaches that at low frequencies, and in this way the response dynamics speed up. These findings can be accounted for quantitatively by a subtractive-feedback model in which there is a linear feed-forward pathway and a feedback pathway whose gain is proportional to the mean level of retinal illuminance.

A family of temporal transfer functions measured at various backgrounds can be fit by an equation of the form

$$H(f,I_o) = A(f)/\{1 + I_o \cdot B(f)\},$$

where $H(f,I_o)$ is the value of the horizontal cell's temporal transfer function (mV/light modulation amplitude) evaluated at frequency f and mean retinal illuminance I_o; A(f) is the transfer function of the feed-forward pathway; and $I_o \cdot B(f)$ is the open-loop gain of the feedback loop.

This work was supported by NIH grants EY01472 and EY00188.

235.14 INFLUENCES OF GRATING CONTRAST AND DURATION ON THE RESPONSES OF CAT RETINAL GANGLION CELLS. Kenneth E. Kratz and James G. May* Louisiana State University Medical Center and University of New Orleans, New Orleans, Louisiana.

In a previous investigation we reported the results of manipulating duration and spatial frequency of a pattern appearance sinewave grating on the responses of cat retinal ganglion cells.[1] Holding contrast constant we found that the latency of the initial excitatory response (time between stimulus onset and response onset) increased with increasing spatial frequency for both X and Y cells, and Y cells had shorter latencies than X cells at low spatial frequencies (<.5 c/d). Latency however, was unaffected by stimulus duration while response duration (time between response onset and response offset) increased with increasing stimulus duration. Also, persistence (time between stimulus offset and response offset) decreased with increasing stimulus duration. In the present study we report the effects on these same measures of manipulating grating contrast while holding stimulus duration constant. We recorded extracellularly the responses of single, optic tract axons in normal cats to the presentation of sinewave gratings of varying spatial frequency (.25,.5,1.0 c/d) and contrast value (.6,.33,.15,.10). A constant space average luminance of 30 cd/m^2 was maintained across the CRT. The gratings were presented for 100 msec at a rate of 2hz and were positioned to evoke the maximal excitatory onset response. Post-stimulus time histograms were obtained for 500 msec following stimulus onset. Average response latencies for both X and Y cells increased with decreasing grating contrast at all spatial frequencies. Also for both X and Y cells, response duration and persistence decreased with decreasing grating contrast. In addition, we determined contrast thresholds for X and Y cells to the presentation of gratings of varying spatial frequency and stimulus duration (25-300msec). These latter measures (determined auditorally) provided estimates of temporal integration for these two classes of retinal ganglion cells. These plots indicate that the critical durations for X and Y cells differ in that the average critical duration for X cells remains constant across spatial frequency but for Y cells increases with increasing spatial frequency.

Supported by NIH grants NS15473 and EY03483 and BRSG grant 2807RR053756-21.

[1]Kratz,K.E. and May,J.G. Measures of latency and persistence of cat retinal ganglion cells to sinewave gratings. Supple. to Invest. Ophthalmol. and Vis. Sci. 24:218, 1983.

235.15 COMPARISON OF PROTEINS TRANSPORTED IN RETINOFUGAL PATHWAYS OF NORMAL ADULT CATS AND RABBITS. A. S. Kelly* and S. A. Schemel*. Div. of Neurobiology, Univ. Calif. San Francisco, S.F., CA 94143.

We have compared the proteins transported via fast transport in the retinofugal pathways of normal adult cats and dutch-belted rabbits, using a combination of axonal transport of [^{35}S] methionine and two-dimensional gel electrophoresis techniques. In initial studies we determined the amount of radioactivity present in optic nerve and tract segments and in target nuclei, the lateral geniculate nucleus (LGN) and superior colliculus (SC), as a function of survival time. The initial wave of radioactivity had arrived at target nuclei with 8 hours of survival in the rabbit, and with 24 hours of survival in the cat. Two-dimensional electrophoresis of nerve, tract and target tissues at these survival times revealed that over 70% of the proteins between 10,000 and 100,000 Daltons and between 3.5 and 7 isoelectric point present in these tissues are the same in the cat and the rabbit. Autoradiograms of these gels indicate which proteins were transported to these loci from the retina, given appropriate controls for blood-borne label and leakage at the target sites (Wagner et al., 1978). Over 75% of the labelled proteins identified in cat tissue were also present in rabbit tissue. In particular, the 3 most heavily labelled proteins described previously in rabbit tissue (Kelly et al., 1980) were common to both species. One of these exhibits microheterogeniety in both species, and is more basic in SC tissue than in LGN tissue, and more acidic in optic tissue than in LGN tissue, in both species. The isoelectric point of this protein does not change as a function of time, and is very reproducible. The differences reported were observed in 17 of 18 rabbits and in 6 of 6 cats. We do not as yet know where this portion is located in axons or their terminals, or what the functional role of such a protein modification in mammalian central visual pathways may be.

235.16 LABELLING OF RETINAL PROJECTIONS IN THE MORMYRID FISH,GNATHONEMUS. G. Làzàr°, S. Libouban° and T. Szabo° (SPON: R.L. Thompson). Dept. Neurophysiologie Sensorielle, Lab. Physiologie Nerveuse, CNRS, 91190 Gif sur Yvette, France.

It was stated by several authors (Lissmann, H.W., J.exp.Biol., 35:156, 1958; McEwan, M.R., Acta Zool., 19:427, 1938; Teyssèdre,C. & Moller, P., Z.Tierpsychol., 60:306, 1982) that weakly electric fish e.g. mormyrids may have a poorly developed visual capacity obviously compensated by an electric sense, specific to these fish. Therefore, the retinal projections were studied in G.petersii using Fink-Heimer, HRP and cobalt-lysin labelling techniques. HRP labelled retinal axons and terminals were visualized by the Hanker-Yates technique, while the cobalt procedure was used according to Làzàr et al., (J.comp.Neurol., 215:108, 1983). The cobalt method always gave a more complete filling of fibers and projections.

The optic nerves do not cross completely in the chiasma. The crossed fibres form a lateral and medial tract. The former covers the lateral surface of the diencephalon and splits into a ventral and a dorsal branch at the rostral end of the tectum opticum. The medial tract is composed of loosely arranged fascicles of axons coursing laterally and dorsally to the lateral forebrain bundle. Its most medial part gives rise to the fasciculus dorsomedialis tractus optici. On the contralateral side optic fibers terminate in five areas, 1. hypothalamus,nucl. suprachiasmaticus (= hypothalamic optic nucl. or preoptic nucl. of other authors), 2. thalamus,nc. opticus dorsolateralis, and nc.geniculatus lateralis, 3. praetectal area: nc. corticalis, nc. comm. post. and nc.pretectalis. The densest projection is found in the nucl. corticalis, whereas only few fibers are seen in the lateral part of the nc. praetectalis, 4. Tectum opticum where the fibers terminate in the stratum fibrosum and griseum superficiale. A few slender fascicles reach this layer also through the str. marginale and str.opticum. 5. Accessory optic nuclei identified in the rostral tegmentum mesencephali. A telencephalic projection through the lateral forebrain bundle was also detected although its terminal field has not yet been determined.

A relatively large ipsilateral optic bundle enters the nc. suprachiasmaticus ventro-medially. Few retinal fibers could be followed into all ipsilateral optic centers except the nc.gen.lat. and the nc.pretectalis.

It may be concluded that the general organization of the visual system in Gnathonemus is similar to that of other teleosts, although identification of the nc.geniculatus lat. and the accessory optic nc. remains uncertain. The less developed projections in comparison to diurnal teleosts may reflect the restrained visual capacity of this fish.

235.17 THE ON CHANNEL AND VISUAL FUNCTION. P. H. Schiller*, J. H. Sandell, and J. H. R. Maunsell. (SPON: B. M. Dawson). Dept. of Psychology, M.I.T., Cambridge, MA 02139

The effects of reversibly blocking the retinal ON channel were examined for several visual functions. 2-amino-4-phosphonobutyric acid (APB) was injected into one eye of rhesus monkeys to produce vitreal concentrations of 200-600uM APB. Concentrations in excess of 400uM effectively blocked the ON channel for 8 to 24 hours as determined by ERG, evoked potential or single unit recordings. Absolute threshold, spatial and temporal contrast, acuity, flicker, color discrimination and visually guided eye movements were examined using either a staircase method or the method of constant stimuli. Each trial was initiated with the appearance of a fixation spot, and the stimuli to be detected or discriminated appeared to either side of this spot. Animals were required either to touch the correct stimulus (detected on a touch panel) or to saccade to it (as assessed with a scleral search coil). Response accuracy and reaction time measures were taken.

Our results show that APB had little effect on high-contrast acuity, critical flicker frequency and color discrimination. Absolute threshold for light increment in the light and dark adapted animal was elevated by 1 and 2 log units, respectively. Spatial and temporal contrast sensitivities were significantly elevated. Saccadic eye movement accuracy to visual targets was unaltered, but there was a significant increase in saccadic reaction time.

The ON and OFF channels originating in the retina make it possible to signal both light increment and light decrement with an excitatory process to the central nervous system. Our results suggest that these two systems (1) provide for high spatial and temporal contrast sensitivity and (2) improve the speed of information processing.

This research was supported by the following grants: NIH EY00676, NSF BNS8019714, and NIH EY02621.

SUBCORTICAL VISUAL PATHWAYS I

236.1 RETINOTOPIC ORGANIZATION OF THE MEDIAL INTERLAMINAR NUCLEUS OF THE CAT. C. Lee*, J. G. Malpeli, H. D. Schwark and T. G. Weyand. Dept. of Psychology, University of Illinois, Champaign, IL 61820.

The purpose of this experiment was to obtain a total map of the medial interlaminar nucleus (MIN) at a level of detail sufficient to resolve the lamination pattern and to allow estimation of the entire range of visual fields represented in each layer. Rather than assembling a map from partial studies, the MIN of a single cat was exhaustively and systematically mapped.

The cat was held in a stereotaxic frame modified to leave the visual field unobstructed. The direction of gaze was determined precisely for each eye by comparing the whole-mounted retinae with the pattern of blood vessels projected onto the screen during the experiment. Electrode passes were made in a rectilinear array, spaced 400 microns antero-posteriorly and 200 microns medio-laterally, spanning the MIN in all directions. Data were taken every 100 microns along each pass. A total of 1568 points were mapped in 102 passes. Of these, 480 points in 65 passes were within the MIN as determined by physiological and histological criteria. Computer reconstructions of these data allowed analysis of the visual field map in coronal, horizontal, and parasagittal planes, and aided in assigning each point to one of the 3 MIN layers following the terminology of Guillery et al. (J. Comp. Neurol., 194: 117-142, 1980).

We confirm physiologically the trilaminar structure of the MIN previously revealed by anatomical methods (Hayhow, W.R., J. Comp. Neurol., 110: 1-64, 1958), as well as the general form of the retinotopic map described by others. Generally, the lowest elevations are found anteriorly, the highest elevations posteriorly, and extreme azimuths ventrally. The representation of the vertical meridian is adjacent to the lateral border of layer A of the LGN. The ipsilateral hemifield representation in layer 3 extends 25 degrees from the vertical meridian. Neither any single layer nor the MIN as a whole represents an entire hemifield. All layers have a very strong bias for the lower visual field. Overall, 68% of the volume of the MIN is devoted to the lower visual field. One striking feature of this map is the absence of significant naso-temporal overlap in any given layer. The range of nasotemporal overlap for the 3 layers is 0-0.5 degrees with an uncertainty of 0.5 degrees.

(Supported by grants NIH RO1 EY02695, NIH KO4 EY00229, NIH T32 EY07005, PHS ST32 GM7143, and grants from the University of Illinois Research Board and Biomedical Research Support Committee.)

236.2 RELATIONSHIPS BETWEEN CELL BODY SIZE AND AXON TERMINAL FIELDS IN SINGLE X- AND Y-CELLS OF THE RETINA AND LATERAL GENICULATE NUCLEUS. A.L. Humphrey, M. Sur and S.M. Sherman, Dept. of Neurobiology & Behavior, SUNY at Stony Brook, NY 11794.

We are using the technique of intracellular injection of horseradish peroxidase to visualize the somata and axon terminal fields of single, physiologically identified X- and Y-cells within the retinogeniculate and geniculocortical pathways of the cat. We hope to determine what relationships exist among such parameters as cell soma size, volume of axon terminal field, and receptive field eccentricity. Another goal is to determine whether X- and Y-cells differ in any of these relationships.

Single axons of LGN cells were recorded in the optic radiations below areas 17 and 18 using HRP-filled micropipettes. Retinal ganglion cell axons were recorded in the LGN or in the optic tract just below the LGN. After each axon was identified extracellularly as X or Y, it was impaled intracellularly and HRP was iontophoresed into it. Post-injection survival time extended from 15-30 hrs. to allow transport of the HRP anterogradely through the axon terminal field and retrogradely to the cell body.

We have analyzed the soma/axon pairs of 10 retinal ganglion cells and 10 cells from the LGN. No clear relationship was seen between soma size and terminal field volume for the X- or the Y-cells. However, among retinal and LGN neurons, there is a moderately strong relationship between a cell's soma size and the number of boutons in its terminal field (r=0.74 and 0.77, respectively). This relationship appears to be stronger for Y-cells at both locations than for X-cells, but more neurons are needed to confirm this. The mean soma size of labeled retinal X- and Y-cells is roughly twice that of those in the LGN, but the LGN cells contain 2-3 times more boutons in their axon terminal fields than do the retinal neurons. Thus, while the number of terminals is related to soma size among cells in the retina and in the LGN, the absolute relationships differ between the two regions. Y-cells of the LGN that project to area 17 exhibit larger terminal field volumes (r=0.9) and greater numbers of boutons (r=0.9) with increasing receptive field eccentricity. The density of synaptic boutons in this group of Y-cells is roughly constant with retinal eccentricity (r=0.9). Geniculate X-cells that project to area 17 and Y-cells that do so to area 18 also show increased terminal field volumes with eccentricity, but it is not yet clear whether their density of boutons remains constant or decreases with increasing eccentricity. Retinal Y-cells, but not X-cells, show an eccentricity-related increase in terminal field volume within the LGN.

Supported by USPHS Grants EY04091 and EY03038.

236.3 THE ULTRASTRUCTURAL SUBSTRATES FOR SYNAPTIC CIRCUITRY OF AN X RETINOGENICULATE AXON. J.E. Hamos, D. Raczkowski, S.C. Van Horn* and S.M. Sherman. Dept. of Neurobiology & Behavior, SUNY at Stony Brook, NY 11794.

We have begun to investigate the ultrastructure of connections made by a single optic tract axon in the LGN. We intracellularly recorded and electrophysiologically identified an axon from a retinal X-cell in the optic tract and iontophoresed HRP into that axon. We then perfused the animal and processed the LGN for electron microscopy. We serially sectioned tissue containing the axon's terminal field, and we reconstructed neurons which were postsynaptic to the labeled terminals from the injected axon.

We have found remarkable selectivity in the contacts from the injected axon. Labeled synaptic terminals repeatedly contact dendrites of some neurons while totally avoiding those of others. However, the pattern of contacts by the X-axon varies on the postsynaptic partner as seen to date in three examples: Cell 1 receives numerous labeled (41%) and unlabeled (59%) retinal inputs, both on its main dendritic shafts and on dendritic appendages. Also, the labeled terminals are preferentially located near the cell body so that labeled inputs form 76% of the retinal terminals within 25μm of the cell body and 29% of those further than 50μm away. Therefore, not only has the injected axon selectively innervated this cell, but it has also selected a particular region of this neuron to contact. Cell 2 receives only unlabeled retinal terminals on its dendrites in the region in which LGN relay cells typically receive retinal input (i.e., within 100μm of the cell body). However, in a secondary retinal recipient zone (150-200μm from the cell body) 29% of the retinal terminals are labeled. Once again, the injected axon has selectively innervated a neuron in a specific region. Cell 3 has several dendritic regions that contain "grape-like clusters" characteristic of certain geniculate cells. In several cases, the injected axon is the sole retinal input to the region. For example, in a cluster of 12 multilobed appendages, there are 16 labeled retinal inputs to the spine or its adjacent dendrite and no unlabeled retinal inputs. These clusters thus seem to be a focal point for retinal input from a single axon.

We have thus far found three distinctly different patterns of input from a single retinal axon. We also found differences in the relationship of nonretinal inputs to these same cells. Further analysis of this and other injected axons will allow us to begin to define the structural details of retinogeniculate circuitry.

Supported by USPHS Grant EY03604.

236.4 A COMPARISON OF A-LAMINAE AND C-LAMINA Y-CELLS IN THE DORSAL LATERAL GENICULATE NUCLEUS OF THE CAT. Joseph Frascella* and Stephen Lehmkuhle* (SPON: Charles Elbaum). Department of Psychology, Brown University, Providence, RI 02912

We recorded extracellularly from isolated Y-cells in the A-laminae and the upper C-lamina of the dorsal lateral geniculate nucleus of the cat and compared their responses to several sine wave grating presentations. Both spatial and temporal contrast sensitivity functions were determined for these cells as well as suprathreshold response functions with 20 and 40% contrast levels using 2 Hz sine wave counterphasing gratings at a number of spatial frequencies.

The results from the spatial contrast sensitivity show that the C-lamina Y-cells were much more sensitive than A-laminae Y-cells at the lowest (0.175 and 0.25 cycles/degree) and highest (3.00 and 4.00 cycles/degree) spatial frequencies and only slightly more sensitive at intermediate spatial frequences. Temporal contrast sensitivity functions revealed that C-lamina Y-cells were more sensitive than A-laminae Y-cells across all temporal frequencies tested. The fundamental response amplitudes of both groups of Y-cells were similar across all spatial frequencies for both suprathreshold contrast levels; however, the second harmonic responses were different. At both 20 and 40% contrast, the C-lamina Y-cells gave larger second harmonic responses across all spatial frequencies with the largest differences at 40% contrast. The second harmonic amplitudes were the greatest at the intermediate spatial frequencies for both populations of cells.

These results indicate clear response differences between A-laminae Y-cells when compared to those in the C-lamina. The largest difference occurs in the second harmonic measures thereby revealing a difference between these populations of Y-cells in subunit contribution. Preliminary analysis of the data has shown a possible difference in response properties of lamina A and A1 Y-cells.

236.5 RESPONSE PROBABILITY OF X- AND Y-CELLS IN THE LATERAL GENICULATE NUCLEUS (LGN) OF THE CAT. A. K. Sestokas and S. W. Lehmkuhle* Dept. of Psychology, Brown University, Providence, RI 02912.

The discharge patterns of LGN neurons vary during repeated presentations of a visual stimulus. We have begun to investigate this variability by studying the effects of stimulus grating contrast and spatial frequency on response probability. Specifically, we analyzed the discharge patterns of LGN X- and Y-cells in anaesthetized, paralyzed cats during repeated 100-msec stimulus presentations. Neurons were tested with vertical, sinusoidal gratings of fixed spatial frequency at 6 values of contrast between 5 and 40 percent, and with gratings of fixed contrast at 6 values of spatial frequency between .17 and 2.0 cycles/degree. Each stimulus was presented 20 times at a rate of 1 presentation per second. A 450-msec period preceding stimulus onset was defined as the baseline period for each stimulus trial. A neural response was defined to have occurred after stimulus onset if 2 criteria were met. First, instantaneous spike frequency had to exceed the mean baseline neural response by at least 2 standard deviations (SD) of the mean baseline instantaneous frequency within 200 msec of stimulus onset. Second, the instantaneous frequency had to remain above this 2 SD window at least 50% longer than it maximally did during the preceding baseline period. The proportion of trials in which a response occurred was then calculated for each stimulus condition tested. The results show that both the spatial frequency and contrast of stimulus gratings influenced the probability of response occurrence. X-cells were more likely to respond at intermediate spatial frequencies than at low or high spatial frequencies. Y-cells were more likely to respond at low than at high spatial frequencies. Moreover, at low spatial frequencies Y-cells were more likely to respond than X-cells, whereas at high spatial frequencies this result was reversed. Finally, response probability increased with increases in stimulus contrast equally for both X- and Y-cells.

The peak responses in these stimulus conditions were measured in 2 ways. First, when peak responses were determined from peristimulus histograms based on the original raw data, a similar pattern of results was obtained for X- and Y-cells as a function of spatial frequency and contrast as that observed for response probability. Second, when peak responses were determined from traces of instantaneous spike frequency, the peak responses of Y-cells generally exceeded those of X-cells for all stimuli tested. These results suggest that response amplitude estimated from conventional peristimulus histograms may be significantly influenced by response probability, which varies as a function of cell type and stimulus condition.

(Supported in part by PHS grants RO1 EY 03524-02 and T32 EY07029)

236.6 DIFFERENTIAL CYTOCHROME OXIDASE STAINING OF OFF- AND ON-CENTER VISUAL CHANNELS IN RETINA AND LATERAL GENICULATE NUCLEUS OF FERRET AND MONKEY. G.H. Kageyama* and M. Wong-Riley (SPON: C. Brown). U. of Calif. San Fran. CA 94143 and Med. Coll. of Wisconsin, Milwaukee, WI 53226.

In certain species of mammals, the functional segregation of OFF-center (OFF) and ON-center (ON) visual channels in the retina (Nelson et al. '78) is preserved within the lateral geniculate nucleus (LGN) as well (Schiller & Malpeli '78; LeVay & McConnell '82). In order to determine whether the ON and OFF visual pathways have different levels of oxidative metabolic activity, sections of retina and LGN from normal ferrets, macaque and squirrel monkeys were reacted histochemically for cytochrome oxidase (C.O.). In the ferret LGN, each of the A laminae is subdivided into inner (A, A1) and outer (A', A1') leaflets (Linden et al. '81), which respectively contain a higher percentage of ON-center and OFF-center geniculate neurons (Zahs & Stryker, personal communication). The outer (OFF) leaflets were notably more reactive for C.O. than the inner (ON) leaflets. Similarly, in the inner plexiform layer (IPL) of the ferret retina, sublamina a (IPL-a; presumed OFF) was more reactive than sublamina b (IPL-b; presumed ON). In the macaque LGN, the dorsal laminae (6 & 5) contain predominantly ON cells, and the middle laminae (3 & 4) contain mainly OFF cells (Schiller & Malpeli '78). While the primate magnocellular laminae (1 & 2) may be the most reactive (Kageyama & Wong-Riley '82), the ON parvocellular laminae (5 & 6) were more reactive than the OFF laminae (3 & 4). The corresponding IPL-b (presumed ON) in the retina was usually more reactive than IPL-a (presumed OFF). These results demonstrate that ON and OFF channels may exhibit different levels of activity. While the OFF channel appears to be metabolically more active in the ferret, the converse seems to be true in the monkey.

In all species studied (see also Kageyama & Wong-Riley '82), the large (presumed Y or Y-like) retinal ganglion cells and LGN neurons tended to be more darkly reactive than medium and small neurons in the same region. Thus, our findings suggest that, in addition to ON- and OFF-channel differences, the Y or Y-like systems may, in general, be metabolically more active than the X/X-like or W/W-like systems.

References:

Kageyama & Wong-Riley ('82) Soc. Neurosci. Abst. 8:208.
LeVay & McConnell ('82) Nature 300:350.
Linden et al. ('81) J. Comp. Neurol. 203:189.
Nelson et al. ('78) J. Neurophysiol. 41:472.
Schiller & Malpeli ('78) J. Neurophysiol. 41:788.

(Supported by NIH Grant: NS 18122)

236.7 ULTRASTRUCTURAL IDENTIFICATION OF SATELLITE INTERNEURONS IN THE RAT DORSAL LATERAL GENICULATE NUCLEUS: AN EM-HRP STUDY. V. M. Montero and G. L. Scott*. Dept. Neurophysiology, Waisman Center, Univ. of Wisconsin, Madison, WI 53706.

Multiple injections of horseradish peroxidase (HRP) were placed in the occipital cortex of rats, including all striate and extrastriate visual areas, to examine with the electron microscope the population of retrogradely labeled and unlabeled cells in the dorsal lateral geniculate nucleus (LGN), for the purpose of ultrastructurally identifying geniculate interneurons. O-tolidine was used as chromogen in the histochemical procedure. EM analysis of LGN showed dense HRP reaction products diffusely distributed in the cytoplasm of virtually all large and medium size geniculate cells, identifying the geniculo-cortical relay cells. The classes of unlabeled cells distinguished in this study are oligodendrocytes, astrocytes, and other small cells whose glial or neural nature is difficult to assess in light microscopic semithin sections. In serial ultrathin sections, these small cells were seen to be presynaptic in somato-somatic synapses with relay cells, and to give origin to presynaptic dendrites. These neurons are cytologically identifiable by their small size, scant cytoplasm, characteristic chromatin clumps in nuclei that are not indented, and by having extended contracts with the cytologically different relay cells. We conclude that these neurons were not retrogradely labeled because of their intrinsic nature in LGN and, because of their common contacts with relay cells, we term them "satellite interneurons".

236.8 FUNCTIONAL CHANGES IN THALAMIC LATERAL GENICULATE NEURONS DURING OSCILLATIONS OF THE SPONTANEOUS RETINAL INPUT FREQUENCY IN CHRONIC CATS. A. Fourment*, J.C. Hirsch* and M.E. Marc* (SPON: M.C. CALVET). INSERM U3, 91 Bd de l'Hôpital, 75634 Paris cedex 13 France.

In lateral geniculate (LGN) relay cells of cats, the maintained discharge of retinal ganglion cells determines monosynaptic composite EPSPs (S potentials of BISHOP et al, 1962: S-pot), subthreshold or generating spikes. Since in adult cats chronic deafferentation causes intense neuronal reorganization, we investigated if the naturally occurring pseudo periodical pauses in retinal discharges changed post synaptic LGN activities during the sleep wake cycle. This was performed by using potassium citrate filled micropipettes to record juxta- and intracellularly S-pot and spikes in chronic cats adapted to head restraint.

During quiet wake and slow wave sleep uncorrelated to any obvious alteration in the behavioral state, transient reductions in frequency of spontaneous subthreshold S-pot occurred for periods lasting 3 to 30 sec. There was a concomitant decreased firing during which bursts predominated but no change in spike height. During these S-pot poor periods the cell membrane was hyperpolarized by 2 to 15 mV, and a depression or reversal of synaptically evoked IPSPs was observed.

Concurrently the characteristics of spontaneous S-pot varied: control subthreshold S-pot displayed an abrupt rising slope, less than 500 µsec, followed by a slow (40 msec) rounded depolarization; control suprathreshold S-pot generated after a few msec one to three spikes which often regained the amplitude of the rounded depolarization; subsequent subthreshold S-pot arising from the rounded depolarization or from a synaptically evoked IPSP yielded a decrease in the amplitude of both the fast rise and the slow return, suggesting an underlying increased conductance. In sharp contrast, during S-pot poor periods most of the few remaining S-pot generated spikes after a delay shorter than 1 msec. Moreover, the amplitude of the fast rising initial phase of the S-pot increased by 18 to 25%, as it did during artificial membrane hyperpolarization applied during control periods. Simultaneously the amplitude and half-width of the rounded depolarization decreased significantly in comparison with control periods and with membrane hyperpolarization elicited during that time.

Thus a transient fall in the number of monosynaptic retinal input triggers a tonic membrane hyperpolarization which enhances the efficiency of the few remaining S-pot to elicit spikes while reducing the amplitude of the slow post depolarization.

(Supported by a CNRS Grant, ATP 033805).

236.9 SPATIAL RELATIONSHIPS OF THE COLLICULAR INFLUENCE UPON LATERAL GENICULATE CELL ACTIVITY IN RABBITS. S. Molotchnikoff, D. Delaunais*, C. Casanova and F. Tremblay*. Département de Sciences biologiques, Université de Montréal, Montréal, P.Q. Canada H3C 3J7.

It is now established that the Superior Colliculus (SC) is involved in the mechanisms of goal-directed movements. For instance, the saccadic intregative model (spatial models[1]) is based on the peculiar organization of the SC which allows for a point-to-point activation of deep saccadic-related neurons by overlying visual cells. This is possible because the dorsal layers of the SC receive retinotopic afferents while the ventral layers seem to correspond with an eye movement map [1]. It also has been shown that the dorsal layers of the SC send fibers to the lateral geniculate nucleus (LGN). Thus, it may be expected that the influence of the SC on the LGN [2] exhibits spatial relationships. The proposed investigations were aimed at confronting this latter hypothesis. In anesthetized and paralyzed rabbits, glass micropipettes filled with 2M NaCl were used to record lateral geniculate cell responses to localized stimuli presented within its receptive field (RF). Stimuli delivered to the geniculate (Flashed spots, LED, bars sweeping across the RF in various directions) were presented in a random sequence. The SC was manipulated in two fashions: a) cells of the SC were excited with an appropriate stimulus positioned within their RF (dual stimulation) or b) collicular cells were inactivated with a microinjection of $CoCl_2$ or Xylocaïne (100 nanol.). The geniculate responses were compared prior to and following collicular excitation or inhibition. To be considered for analysis the variations of the geniculate responses must be greater than 20%. So far the results can be summarized in the following manner. Collicular inactivation with cobalt produced a decrease of all tested geniculate OFF-cells (5). By contrast all ON and ON-OFF cells (6) had their responses enhanced. The dual stimulation protocole resulted in a decrease of only two OFF cells, whereas (16) ON and ON-OFF cells had their discharges diminished. Finally, the influence of the SC upon LGN ON and ON-OFF units declined as the distance between the collicular and the geniculate RF increased. This latter trend seems to indicate that the collicular impact upon geniculate activity is stronger when both RFsare in register.

[1] SPARKS, D.L. and MAYS, L.E. J. Neurophysiol. 49, 45-63, 1983.

[2] MOLOTCHNIKOFF, S. and LACHAPELLE, P. Exp. Brain Res. 40, 221-228, 1980.

Supp. by CRSNG and FCAC

236.10 DISINHIBITION OF DORSAL LATERAL GENICULATE NEURONS FOLLOWING LESION OF THE THALAMIC RETICULAR NUCLEUS. G. A. Marks, A. Stabrowski* and H. P. Roffwarg. Dept. of Psychiatry, University of Texas Health Science Center, Dallas, TX 75235.

The excitability of relay cells in the rat dorsal lateral geniculate nucleus (dLGN) is depressed following a shock to the optic tract (OT). Paired shocks less than 100 msec apart will fail to elicit a response to the second shock. Electrophysiological and anatomical evidence strongly implicate the visually responsive cells of the thalamic reticular nucleus (TRN) as the responsible mechanism acting through feed-back or feed-forward inhibition of dLGN relay cells. Sumitomo et al[1] have reported the results obtained from one rat showing that electrolytic lesion of the visually responsive TRN shortens the post-excitatory recovery of relay cells as measured by double-shock OT stimulation. In confirmation of these results, we report that electrolytic destruction of the caudal pole of the TRN leads to a disinhibition of neuronal elements in the dLGN in response to the second of a paired shock to the OT.

Long Evans hooded rats under chloral hydrate anesthesia were bilaterally implanted with 122.5 um nichrome wire in the dLGN and wire with 1.0 mm of insulation removed in the caudal TRN. A bipolar stimulating electrode was placed near the OT at the level of the chiasm. A double shock, 100 msec apart, delivered to the OT would always elicit a short latency multiple unit response to the first shock and never to the second. Increasing amounts of RF current was passed to the TRN electrode until either a response to the second shock would appear or activity was lost in the dLGN. Utilizing this method, success at eliciting a response to the second shock is greater than 50% and in all successful experiments histology revealed the lesion to have destroyed the caudal pole of the TRN.

Figure: Oscilloscope tracings of activity recorded from the dLGN. Large amplitude stimulus artifact indicates 2v shock to OT. Shocks are 100 msec apart. 1. pre-lesion, no response to second shock. 2. post-lesion, response to both shocks.

These data support the role of the visually responsive cells of the TRN in the post-excitatory inhibition of dLGN relay cells.

[1] Exp. Neurol., 51:110-123 (1976)

236.11 THE RELAY OF INFORMATION FROM DORSAL LATERAL GENICULATE NUCLEUS (LGNd) TO VISUAL CORTEX IN THE AWAKE RABBIT. H. A. Swadlow and T. G. Weyand. Dept. Psychology, Univ. Conn., Storrs, CT 06268

The inherent stability of the rabbit eye was exploited to enable receptive field analysis of LGNd neurons and optic tract axons in the awake, unparalyzed state. Axonal properties were also studied. The eye monitoring system we generally used was accurate to 1/3° (early experiments) or 1/5° (later experiments). In some experiments, however, we reflected a low intensity laser off of a mirror cemented to the eye. Using this system (accurate ± 2 min.) to test eye stability, we commonly observed eye position to remain within a range of 1.0° for 4-5 minutes and, in some cases, for periods in excess of 10 minutes. Such stability is comparable to that seen in awake monkeys trained to fixate.

The receptive fields of approximately 85% of LGNd neurons were concentrically organized. This is a higher value than previously reported in this species and we argue that many of the uniform cells previously described in rabbit LGNd show, in our experimental situation, a clear surround mechanism. Most of the remaining neurons were either directionally selective (7%) or motion/uniform (7%). Concentric sustained cells were found predominently near the representation of the visual streak while concentric transient cells were more common in the upper visual field. In the region of the visual streak, receptive field diameters of sustained cells were somewhat smaller (median = 1.7°) than those of transient cells (median = 2.3°). Both sustained and transient cells showed primarily non-linear spatial summation. As reported previously (Swadlow, H. A. and Weyand, T. G., Neurosci Abstracts, 8: 207, 1982), non-visual sensory stimuli that resulted in EEG arousal had a profound effect on the duration of the response of most sustained concentric neurons. Such arousing stimuli usually had little or no effect on the spontaneous firing level of the cell in the absence of visual stimulation. Arousing stimuli had no effect on the response of sustained, concentric optic tract axons.

Approximately 77% of LGNd neurons tested (127/165) were antidromically activated following electrical stimulation of the visual cortex. All receptive field classes of LGNd neurons projected axons to the visual cortex. Thus, no evidence for a separate class of intrinsic interneuron was obtained. While concentric sustained neurons conducted somewhat more slowly than did transient neurons, antidromic latencies were quite similar for all classes of cells studied.

236.12 FUNCTIONAL CHARACTERISTICS OF PRE- AND POSTSYNAPTIC FIELD POTENTIAL RESPONSES IN THE RETINOGENICULOSTRIATE PATHWAY OF HOODED RAT. D. Impelman*, C.L. Lear*, R. Wilson* and D.A. Fox. Coll. of Optometry, Univ. Houston and Div. Tox., UTMSH, Houston, TX.

Previously we examined the effects of lead on field potential responses in the retinogeniculostriate pathway of hooded rat (Neurosci. Abs. 8:81,1982). Differences in chronaxies and conduction velocities for the high (H) and middle (M) conduction groups and a depression of geniculostriate recovery cycle (RC) were found. Therefore we further characterized the functional properties of pre- and postsynaptic components as a tool to assess X and Y population responses in rat visual system. Optic nerve fibers in H and M groups project to fast and slow P cells in dLGN which project to cortical areas 17 and 18 and are probably correlates of X and Y cells in cat (J. Physiol. 315:69,1981). Presynaptic responses of H and M were recorded from the optic tract in response to stimulation of optic chiasm (OX) and were identified by their response latencies, chronaxies, frequency following and antidromic responses. Orthodromic RCs for H and M groups were characterized by their refractory, supernormal and subnormal periods. The minimal ISI at which a conduction group was recorded for group I was 0.52-0.71 ms and 0.69-0.95 ms for groupII. Standard plots of stimulus intensity functions showed that maximal response amplitudes for H and M functions were reached at approx. 6.4-8.0 times threshold for group I and 13.3-17.3 times threshold for group II. Stimulus intensity and RCs for geniculate responses differ from their presynaptic inputs mainly in their inhibitory interactions which produce a prolonged and sometimes cyclic recovery for both fast and slow P cells. In our recordings of responses in area 17 to OX stimulation, the pre- and postsynaptic components of P cell input characterize the early part of the waveform. Postsynaptic responses to fast and slow fiber input are identified by their response latencies, frequency following and RC responses. The late P cell discharge, at 150-200 ms in fast P cells and at 250-400 ms in slow P cells, occurs as a characteristic increase in excitability in their postsynaptic RC responses. The early depression of postsynaptic RC responses occurs in absence of any correlated changes in presynaptic RC and suggests that cortical depression may be mediated by postsynaptic inhibition as in cat (Exp. Neurol. 21:424,1968). Although the functional characteristics of retinostriate projections of the two conduction groups are more similar in rat than cat (J. Physiol. 121:415,1953 and 150:214,1960) their differences are characteristic. Further information concerning their cortical interactions in area 17 and 18 are the subject of present investigation. Supported by ES 07090 and ES 03183 (DAF).

236.13 CYTOARCHITECTURE AND AFFERENT CONNECTIONS OF NUCLEUS LATERALIS POSTERIOR IN GROUND SQUIRREL. N. Lugo-García and E. Kicliter. Lab. of Neurobiology and Dept. of Anatomy, Univ. of Puerto Rico Sch. of Med., San Juan, PR 00901.

Nucleus lateralis posterior (LP) of the ground squirrel (Spermophilus tridecemlineatus) is a large, wedge-shaped structure considered to be homologous, in part, to the primate pulvinar. We were interested in determining whether LP contains subdivisions, as it does in related species. Our methods included study of LP's cytoarchitecture and connections.

The cytoarchitecture was studied in Nissl-stained sections. Differences were found in the soma sizes and distribution of neurons in rostral and caudal sectors of the nucleus. The rostral portion of LP was observed to consist of medium-sized (5-10 um diameter) cells often grouped in clusters. The caudal sector contained larger neurons (10-17 um diameter) which were more evenly distributed.

Afferent projections to LP were determined by making small iontophoretic injections of horseradish peroxidase into various parts of the nucleus. After 24 or 48 hour survival times the animals were perfused intracardially with aldehydes and brain sections were processed histochemically according to the method of de Olmos and Heimer (J. Comp. Neur. 181: 213-244, 1977). On the basis of connections with the superior colliculus and visual cortex, LP of this species consists of three subdivisions. The rostral portion can be subdivided into rostrolateral and rostromedial sectors. The caudal portion cannot be further subdivided on the basis of connections. Only the rostrolateral and caudal sectors of the nucleus received projections from the superior colliculus. These projections originated in the lower half of the stratum griseum superficiale. On the other hand, all three subdivisions of LP received visual cortical input. Rostrolateral LP received projections from areas 17, 18, 19 and temporal cortex; rostromedial LP from areas 17, 18 and 19; and caudal LP from area 19 and temporal cortex. The cell bodies of all visual cortical afferents were located in lamina V and the upper part of lamina VI.

Thus, differences in cytoarchitecture and afferents evidence the existence of three subdivisions of ground squirrel LP. The organization of ground squirrel LP is similar to that reported for grey squirrels (Robson and Hall, J. Comp. Neur., 173: 355-388, 1977), golden hamsters (Crain and Hall, J. Comp. Neur., 193: 351-370, 1980) and the Chilean rodent Octodon degus (Kuljis and Fernández, Brain Res., 234: 189-204, 1982). The present and previous results further suggest that LP has more than a single role in the processing of visual information.

Supported, in part, by USPHS Grants NS-07464 and GM-07718.

236.14 MECHANISMS FOR RESPONSES TO MOVEMENT-IN-DEPTH IN THE LATERAL PULVINAR OF THE MACAQUE. D. Burman, L.A. Benevento and G. Felsten. Dept. of Anatomy, Univ. of Illinois at Chicago, Health Sciences Center, 808 S. Wood, Chicago, IL 60612

Our previous work has shown that some units in the lateral pulvinar of paralyzed macaques respond to movement in depth, and also to expanding and contracting discs on a tangent screen. The possible bases for these responses have been studied in greater detail. Monocular responses indicate that binocular interaction is not necessary to evoke a response to movement in-depth. Disparity tuning curves, however, suggest that a unit which responds best to an approaching object gives a maximum response to an object in front of the plane of focus. Careful analysis of receptive field organization indicates that the location in the visual field and the sign of response (i.e., excitation or inhibition) to a tangentially moving bar or an expanding circle in the plane of focus, correspond well with the size and location of the expanding or contracting solid spot (disc) that evokes maximum response. Use of radial moving bars seldom if ever increased the magnitude of response. Stimulus intensity was also an important factor. Changes in whole-field intensity or that of discrete spots of light evoked a response that was intensity-dependent, but consistent with the response to movement in-depth or an expanding/contracting disc. For example, a unit which responded to an approaching object (or a dark expanding disc) might respond with excitation to a flash of dim light and inhibition to a flash of bright light; it might also respond to a stationary stimulus changing intensity. The size of the stimulus was also important; for example, a flashing large dark spot would evoke a larger response than a flashing small spot. It therefore appears that at least some pulvinar units detect 3-dimensional movement in-depth by translating information from several 2-dimensional stimulus cues, such as location, size, and brightness, as well as using 3-dimensional cues such as disparity. In this way, monocular as well as binocular cues could give the animal information about movement in-depth. (Supported by EY 2940)

237.1 EVIDENCE FOR PROJECTIONS TO THE SUPERIOR COLLICULUS FROM THE HYPOTHALAMUS AND ZONA INCERTA IN THE CAT. R.W. Rieck and J.T. Weber. Dept. of Anatomy, Tulane University Medical School, New Orleans, LA. 70112

Previous studies have suggested that the hypothalamus and zona incerta project to the superior colliculus (Harting et. al., '79; Edwards et. al., '79). The present study determines the specific origin of this hypothalamotectal projection using the retrograde transport of a lectin bound peroxidase tracer (WGA-HRP). Electrophoretic injections of WGA-HRP were placed unilaterally within the superior colliculus. Animals survived 48 hours, after which they were processed for routine HRP histochemistry. Following large injections of WGA-HRP that include the stratum griseum superficiale (SGS), the stratum opticum (SO) and the stratum griseum intermediale (SGI), extensive retrograde labeling is localized within the zona incerta and the dorsal hypothalamic area (DHA). Scattered cell labeling also is noticed within the lateral hypothalamic area (LHA) and the medial hypothalamic nuclei of the tuberal region, including the dorsomedial nucleus (DM). Labeled neurons within the zona incerta are observed to be medium and large fusiform cells. The labeled neurons within DHA are exclusively small spherical cells that routinely form relatively densely packed clusters that are directly dorsolateral to the apex of the third ventricle. In contrast, the retrogradely labeled neurons within the LHA and medial hypothalamus include widely dispersed small spherical cells and medium sized multipolar neurons. These latter neurons are not apparently restricted to specific nuclear boundaries, however, there appears to be larger numbers of labeled neurons within the medial hypothalamic nuclei. In contrast to the results obtained with large injections, restricted injections of WGA-HRP within either the SGS or the SO result in negligible labeling in either the zona incerta or the hypothalamus. This finding is interpreted to suggest that the SGS and the SO are not the major tectal recipient layers of the projections that arise in the hypothalamus or zona incerta. In conclusion, these results suggest that the zona incerta and hypothalamic projections to the superior colliculus arise from uniquely different groups of neurons, and further, that the hypothalamotectal projection has a preferential termination within SGI as indicated previously by anterograde techniques (Harting et. al., '79).

Supported by NIH grants EY03731 and BRSG 531830.

237.2 'TEMPORAL CODING' FOR PATTERN VELOCITY BY MOVEMENT SENSITIVE VISUAL CELLS IN CAT SUPERIOR COLLICULUS. G. Mandl. Aviation Medical Research Unit, Dept. of Physiology, McGill Univ., Montreal, Canada H3G 1Y6.

This study attempted to relate the temporal characteristics of unit discharge patterns, to the presence or absence of moving visual stimuli within unit receptive fields. METHODS. Spike data were recorded extracellularly from the superficial and intermediate layers of the superior colliculi of unanaesthetized paralyzed pretrigeminal cat preparations. Visual stimuli consisted of small luminous bars (1-4° x 0.2-0.8°) moving under computer control at uniform velocities (2-160°/s) across a CRT screen. Interval histograms, plotted from data acquired at sampling times of 0.2-1 ms, were used to estimate the probability distributions of interspike intervals RESULTS. Spike activities in all tested cells were characterized by two dominant statistical processes: (1) A fast process (FP), consisting of brief irregular high-frequency spike bursts, whose intraburst interval distributions suggested a truncated Poisson process with dead-time (refractory period) and a long 'tail' decaying exponentially from a short (<4 ms) modal interval. (2) A slow process (SP), with periods of near-regular firing (noisy pacemaker), characterized by approximately bell-shaped interval distributions with longer modal intervals (15-50 ms). By relating the interplay of these two processes to external stimulus conditions, each cell could be assigned to one of three functional groups: Group A. Unstimulated activity was dominated by SP. Stimulation with moving pattern caused emergence of FP. An increase in pattern velocity was accompanied by a progressive increase in the FP/SP ratio, without significant changes in the duration of the two respective modal intervals. At one given ('optimal') pattern velocity, cell activity became dominated by FP. Group B. Unstimulated activity was dominated by FP. Stimulation with moving pattern caused complete switch to SP whose modal interval length then varied inversely with pattern velocity. Group C. For all conditions, FP and SP co-existed (double-humped interval distribution). The length of SP modal interval was inversely related to pattern velocity. The length of FP modal interval remained fixed for all pattern velocities, but the time constant of FP exponential decay frequently varied inversely with pattern velocity. Thus, the dominant response of collicular cells to pattern movement does not seem to consist of simple frequency modulation of 'spontaneous' carrier-type 'background' activity. Rather, the present observations suggest that different collicular cells encode the presence, and the velocity, of moving visual stimuli by switching and combining a number of characteristic, stereotyped temporal patterns of discharge.

(Supported by the Canadian Medical Research Council)

237.3 TIME COURSE OF MULTIMODAL RESPONSE ENHANCEMENT IN CAT SUPERIOR COLLICULUS NEURONS, J.W. Nemitz, M.A. Meredith and B.E. Stein, Dept. of Physiol.& Biophys., Med. Coll. Va., Richmond, VA 23298.

Visual, auditory and somatosensory inputs converge on cells in the intermediate and deep laminae of the superior colliculus (SC). These cells respond to combinations of sensory stimuli (combined-modality tests) in a manner that may not be predictable on the basis of their responses to each stimulus individually (separate-modality tests). The 'multimodal interactions' fall into two functional categories: response enhancement and response depression. The present investigation examined the time course of response enhancement.

Experiments were conducted on 14 cats chronically prepared for extracellular recording. Each animal was anesthetized with Ketamine HCl, paralyzed and then artificially respired with a mixture of 75% N_2O and 25% O_2. Visual stimuli consisted of flashed or moving bars of light and auditory stimuli consisted of white noise bursts lasting 100-300 msecs. Each cell was first presented with a series of separate-modality tests consisting of 16 repetitions of each sensory stimulus. These same stimuli were then paired and presented simultaneously 16 times. A multimodal interaction was considered to have occurred if there was a significant ($p<.05$) increase in the number of impulses elicited (this enhancement effect varied from a 28-900% increase in the number of impulses evoked). The durations of the response trains evoked in each cell was normalized so that the duration of the response to the most effective separate-modality stimulus represented a value of 100%. Thus, the time course of the responses among different cells to separate- and combined-modality tests could be compared.

A total of 49 multimodal neurons was identified, of which 22 exhibited enhanced responses when visual and auditory stimuli were presented simultaneously. Typically a cell responded to either stimulus presented alone with a brief burst of discharges at the onset of the discharge train and discharge rate decayed rapidly thereafter. When the stimuli were combined a similar pattern occurred, but clearly the discharge rate was higher throughout most of the discharge train. Unexpectedly, however, the greatest percentage of response enhancement did not correspond to the period in which the maximal response rate was evoked. Rather the greatest increase in discharge frequency produced by combining stimuli occurred only after approximately 40% of the discharge train had already elapsed and response rate would normally have decreased markedly to either stimulus alone. Apparently response enhancement is limited during the period in which optimal response rates normally occur to either stimulus alone.

Supported by grants EY 04119 and BNS 8209857.

237.4 Descending Efferents of the Superior Colliculus Relay Integrated Multimodal Information, M.A. Meredith and B.E. Stein, Dept. of Physiol. & Biophys., Med. Coll. Va., Richmond, VA 23298.

Activity in the superior colliculus (SC) is related to the orienting of sensory receptors (eyes, pinnae, head) toward a sensory stimulus. Cells in the deeper SC laminae are known to connect with brainstem motor and premotor areas which are involved in the generation of these movements. While many of the efferent SC fibers projecting to these regions are carried in the Lateral Efferent Tract (LET) or Medial Efferent Bundle (MEB), little is known concerning the sensory properties of efferent colliculus neurons. The present study was initiated to determine the responses of such cells to sensory stimuli.

Stimulating electrodes were stereotaxically placed in the LET and MEB of each of 7 cats. Routine paralysis, respiration and recording techniques were used. Cells in the SC were identified by their spontaneous activity or by their responses to visual, auditory and/or tactile stimuli. Once isolated, a cell was tested for antidromic activation from either set of stimulating electrodes (70-300 uA, 0.1 ms pulse). Criteria for antidromic activation: 1) < 0.2 ms latency variation, 2) following frequency > 300 Hz, and 3) threshold current < 300 uA. Whether antidromically activated or not, all cells were evaluated for their responses to separate-modality and combined-modality stimulation.

Of the 115 cells identified in the deeper SC laminae, 48.7% (n=56) were antidromically activated by one (LET=10; MEB=33) or both (LET+MEB=13) sets of stimulating electrodes. The vast majority of these antidromically activated cells (51/56; 91%) were responsive to sensory stimuli (visual=48.3%; auditory=31.5%; tactile =20.2%) and the majority (32/51; 62.7%) received multimodal inputs. Often, the multimodal nature of a cell could only be determined by its response to combined-modality stimulation. No differences between responses to separate-modality and combined-modality tests were apparent in 77.9% (n=46) of the neurons unresponsive to efferent tract stimulation (n=59). The remaining non-efferent cells (n=13) were unaffected by sensory stimuli. Neurons identified as efferents occurred with similar frequency in the intermediate and the deep laminae; and there was no apparent laminar segregation with regard to multimodal properties.

Since these efferent tracts connect the SC to premotor areas of the brainstem, multimodal convergence on efferent SC cells appears to constitute the neural mechanism by which sensory information influences the motor behaviors dependent upon the SC.

Supported by Grants EY 04119 and NS 06838.

237.5 VISUAL DESYNCHRONISATION OF EEG IMPAIRED BY LESIONS OF SUPERIOR COLLICULUS IN RAT. P. Dean, P. Redgrave* and L. Molton*. Dept. of Psychology, Sheffield Univ., Sheffield, S10 2TN, U.K.

Rats with lesions of the superior colliculus fail to orient to novel visual stimuli in the peripheral field. It is unclear whether this deficit arises because the animals have difficulty in producing specific orienting movements, or because they fail to detect or notice the stimuli. We therefore examined the effects of collicular lesions on a response to a novel stimulus that did not involve specific movements, namely desynchronisation of the cortical EEG.

Hooded Lister rats were tested in a small isolated chamber with an overhead mounted 6w fluorescent tube. Their EEGs were recorded with bipolar surface-to-depth electrodes, implanted into frontal and visual cortex. On-line analysis of EEG was used to present stimuli upon the appearance of continuous (15 sec) high-amplitude low-frequency activity. Light flashes of increasing duration (from 20 msec in 40 msec steps) were presented until either the EEG desynchronised for at least 5 sec, or flash duration reached 380 msec.

Irrespective of electrode location, both normal animals and animals with control lesions of cerebral cortex overlying the superior colliculus showed EEG desynchronisation to stimuli within the range studied (12 electrodes in 6 animals, median duration = 60 msec). In contrast, at 13 of 14 electrodes tested in 7 animals with lesions of the superior colliculus, desynchronisation was not observed even for the 380 msec flash, although in a subsequent test some evidence of desynchronisation was observed to a 1 sec flash from a high intensity floodlight. The collicular impairment was not the result of: (1) eyelid closure, because a 380 msec flash, delivered only when an observer close to the animal was certain the eyes were open, failed to desynchronise the EEG; (2) a nonspecific deficit in arousal, because no comparable collicular deficit was found with 20-100 msec bursts of overhead white noise; (3) failure to produce observable orienting movements, because no such movements were apparent when the EEGs of control animals desynchronised.

These results support the view that lesions of the superior colliculus in rats do not merely affect specific orienting movements, but rather impair the ability to detect or notice certain kinds of novel visual stimulus. The results also suggest, in conjunction with the observation that low intensity electrical stimulation of the superior colliculus can desynchronise the EEG (Olds and Peretz, EEG Clin. Neurophysiol., 12:445, 1960), that the superior colliculus may be directly involved in alerting cerebral cortex to such stimuli.

Supported by SERC grant GR/B/24707.

237.7 VISUAL ORIENTING RESPONSES FOLLOWING SELECTIVE REDUCTION OF OPTIC INPUT TO AOS AND/OR OPTIC TECTUM IN FROG. K.V. Fite, D. Hayden*, N. Montgomery & L. Bengston*. University of Massachusetts, Amherst, Massachusetts, 01003.

Recent neuroanatomical studies in anurans have revealed a rich complexity of interconnections among mesencephalic visual areas. For example, the nucleus of the basal optic root (nBOR) is the retinorecipient nucleus of the accessory optic system (AOS) and projects primarily to the pretectal nucleus, which is reciprocally connected with the optic tectum. In addition, nBOR has interconnections with the adjacent, medial tegmental region (peri-nBOR), the oculomotor complex, and nMLF and brainstem nuclei. Contrary to earlier reports, we have demonstrated that nBOR plays a relatively minor role in horizontal optokinetic nystagmus (hOKN), while the pretectal nucleus appears to be more crucially involved in mediating hOKN in frog, as in other vetebrate species.

C. J. Herrick (1948) was among the first to suggest that retinal excitation via the largest myelinated optic fibers may produce a "generalized nonspecific effect" which comes to motor expression, first, through the basal optic tract (BOR). Reduction or removal of retinal input to nBOR might thus be expected to alter the timing of certain visually guided behaviors, such as the rapid visual orientation to live prey seen in many anurans. In order to evaluate this hypothesis, either partial-to-complete transections of BOR, direct lesions of nBOR, or localized retinal deafferentation of the optic tectum were produced in Rana pipiens. Extensive pre- and post-surgical visual orientation response-latency functions (VORLs) were obtained using live prey presented randomly at each of 8 equidistant, circumferential locations around the subject's body. The location and extent of optic deafferentation was subsequently evaluated using anterograde transport of HRP. The results indicate that complete BOR transection or nBOR lesions produce consistent, large increases in VORLs at all prey locations, with the largest latency increases occurring for the lateral, monocular and posterior visual field positions. Localized deafferentation of optic tectum and/or partial transection of BOR yielded much smaller latency increases. Prey-catching behaviors were otherwise unaltered and virtually indistinguishable from normal. These data suggest that the AOS conveys an important aspect of visual information which may serve a unique priming or alerting function in those mesencephalic visuomotor circuits which mediate rapid orienting responses in anurans. Whether or not other visuomotor behaviors are also affected by AOS deafferentation remains to be determined. (Supported by NSF BNS-8209026).

237.8 ANATOMICAL AND ELECTROPHYSIOLOGICAL CHARACTERISTICS OF THE NEURONS IN THE FROG TECTUM RECEIVING OPTIC INPUTS. R.A. McCrea and P. Grobstein, Dept. of Pharm. & Physiol. Sciences, Univ. of Chicago, Chicago, IL. 60637

We have begun a study of tectal organization based on intracellular recording and HRP injection in Rana pipiens. Animals were anesthetized with tricaine and had stimulating electrodes placed on the optic nerve. The field potential evoked in superficial tectal layers in response to optic nerve stimulation consisted of 2 negative peaks with latencies of approximately 2 and 5 msec., and two broader positive peaks of latencies approximately 10-12 and 20-30 msec. Spikes recorded from large diameter optic axons had latencies of 2-5 msec. In addition to a local area of dense terminals in the tectum, the axons collateralized in the pretectal nucleus as well as along the course of their trajectory through the tectum.

The earliest synaptic potentials evoked in tectal cells had latencies ranging from 2 to 40 milliseconds. Short latency EPSP's (less than 10 msec.) were more frequently seen in recordings above layer 6 than below. In and below layer 6, we encountered cells which either had no observable synaptic response or in which the earliest response was a long latency IPSP (40 msec. or more). Some deep cells and virtually all superficial cells responded to optic stimulation with EPSP's at 40 msec. or less.

We injected cells having excitatory synaptic inputs with latencies of 40 msec. or less. To date we have recovered such cells in layers 2, 5, 6, 7, 8, and 9. All cells had processes in retinal recipient layers: the arborizations were in some cases quite restricted both horizontally and vertically and in other cases quite widespread. Many cells appeared to lack clear axons or had axons ramifying only locally. Other cells, including cells both above and below layer 7 had axons which left the tectum. Many of these axons collateralized significantly along the course of their way out of the tectum.

While all cells had dendrites in retinal recipient layers, there was substantial variation among cells in synaptic latency. This almost certainly reflects both heterogeneity in optic fiber input and processing within the tectum. The more frequent recording of short latency inputs above layer 6 as well as the reduced frequency of observed excitatory inputs below layer 6 indicates that a functional subdivision into superficial and deep tectal layers may characterize tectal processing in anurans as in mammals. Finally the frequent collateralization observed along axon trajectories suggests that substantial horizontal as well as vertical interactions characterize tectal organization.

Supported by PHS EY-01658 and the Brain Research Foundation.

237.9 DENDRITIC AND AXONAL MORPHOLOGY OF TECTAL-PROJECTING NEURONS IN THE ISTHMUS REGION OF A TURTLE, PSEUDEMYS SCRIPTA. M. Sereno. Committee on Neurobiology, Univ. of Chicago, Chicago, IL 60637.

The tectum receives a well-known topographic retinal input to its superficial layers. Relatively little, however, is known about the cellular morphology of other inputs. Therefore, the dendritic and axonal morphology of tectal-projecting neurons in two isthmus region nuclei was examined using HRP injections and serial-section reconstruction techniques.

As in other vertebrates, there is in turtles a retinotopically organized nucleus (magnocellular nucleus isthmi caudalis, Imc) at the caudolateral pole of the tectum. Imc neurons have restricted dendritic fields and are arranged on a shell-shaped map surface. Their dendrites bear fine filamentous appendages with leafy, en passage varicosities. Cases with two punctate HRP injections in the tectum show that Imc neurons projecting to different tectal loci have non-overlapping dendritic fields. The putative axons of Imc neurons travel just below the superficial gray and turn upwards, giving off cylindrical, vertically-oriented arbors of several thousand boutons. Single arbors are about 100 μm in diameter and extend from the central gray (where strings of large boutons have a reticular appearance) up into the superficial gray (where strings of medium-sized boutons appear in vertical arrays that turn horizontally just below the stratum opticum) and finally into the stratum opticum (where less dense vertical arrays reappear). Each arbor covers less than 0.1% of the tectal surface.

Just rostral to Imc is the magnocellular nucleus isthmi rostralis (Imr) which, unlike Imc, has a non-topographic tectal input and a non-topographic projection back to the tectum. Back-labeled Imr neurons have robust dendrites that arborize in the stream of axons that leave the tectum in the ventral tectobulbar path. Their dendritic fields are large relative to the nucleus and their dendrites bear no appendages. Imr axons are emitted ventrally and then turn up into the tectum where they course under the superficial gray, emitting strings of large boutons. In contrast to Imc axons, Imr axons cover a much larger area (10 - 40%) of the tectum with a much less dense, but patchy, terminal distribution.

These results suggest that the superficial and central layers of the tectum both participate in topographic as well as non-topographic feedback loops with structures outside the tectum. (Supported by PHS Grant NS 12518).

237.10 CHANGES IN RECEPTIVE FIELD ORGANIZATION AND VELOCITY TUNING IN TURTLE TECTAL NEURONS FOLLOWING LASER IRRADIATION. Robbins, D.O., Westgate, T.M.*, Zwick, H.*, Bloom, K.R.* and Schuschereba, S.T.* Department of Psychology, Ohio Wesleyan University, Delaware, OH 43015 and Division of Biorheology, Letterman Army Institute of Research, San Francisco, CA 94925.

The receptive field organization of cells in the turtle optic tectum are organized in a complex manner without clear evidence of traditional center-surround relationships. Exposure of these cells to low level laser irradiation (514 and 633 nm) did not produce obvious spectrally selective losses. The overall effect was to uniformly reduce the cell's sensitivity across its entire visible spectrum. Generally, the more intense the exposure, the stronger its effect on the responsiveness of the cell.

Laser irradiation of the center only, periphery only or the entire receptive field produced a similar overall constriction of the field. With intense irradiation, the entire field would constrict until the cell was no longer responsive to light stimulation. With less intense laser exposures, the initial response of the cell was an expansion followed in time by a gradual reduction of the field diameter.

When receptive fields were mapped with moving targets of varying velocities (0 to 100 deg/sec), receptive field expansion was noted for fields plotted with faster moving stimuli. In the long end of the spectrum this effect was monotonic with velocity but with intermediate wavelength stimuli, the effect was much more nonlinear. As velocity was increased, peaks in the intermediate spectrum became more pronounced. Repeated exposure to visible laser light reduced receptive field size across the spectrum when moving as well as stationary targets were used. Maximum effects, however, were found for fields mapped with higher velocity stimuli, suggesting perhaps involvement of neural processing beyond the receptor level.

At the light microscopy level, minimal alteration of retina tissue was observed with even the maximum dosage level used. The observed laser effects suggest that either the coherency or the narrow bandwidth of laser light may artificially elicit activity not commonly observed with more traditional incoherent light sources and may overdrive the neural transmission system.

237.11 POSSIBLE NEURONAL CIRCUITS IN THE PIGEON'S OPTIC TECTUM : AN INTRACELLULAR RECORDING AND LABELING STUDY. O. HARDY*, N. LERESCHE* and D. JASSIK-GERSCHENFELD*(SPON : European Neuroscience Association). Lab. de Psychophysiologie Sensorielle, Université Pierre et Marie Curie. Paris, France.

Excitatory and/or inhibitory effects from the optic nerve have been described in the pigeon's optic tectum by early extracellular studies (Holden, A.L., J. Physiol., 194:91, 1968 ; Robert, F. and Cuenod, M., Exp. Brain Res., 9:123, 1969 ; Bagnoli, P., Franchesconi, W., Magni , F., Brain, Behav. Evol., 16:19, 1979). In order to identify the neuronal circuits involved on such effects, intracellular recordings were obtained from 65 tectal cells using micropipettes filled with Horseradish peroxidase (HRP). The contralateral optic nerve (ON) was electrically stimulated. In addition, the visual telencephalon or wulst (W) and the opposite optic tectum (OT) were also stimulated. Most tectal neurons responded to ON stimulation either with an EPSP or with an EPSP-IPSP sequence ; few cells responded with a pure IPSP. The polarity of the IPSPs was easily reversed by small intracellular hyperpolarizing currents or by intracellular injection of Cl^- ions. IPSPs were also obtained from W and OT stimulation. Latency measurements show that IPSPs to ON are not due to the transynaptic activation of either W or OT ; most likely such IPSPs are transmitted via intratectal inhibitory circuits of both feedback and feedforward type.

After being intracellularly studied 20 cells were labeled by iontophoretic injection of HRP. Pure EPSPs to ON were recorded from different cell types, i.e., radial, stellate and horizontal cells. The bodies of such cells were located in sublayers IIb, c, i, j. EPSP-IPSP sequences were recorded from radial cells whose bodies were distributed in sublayers IIg, i and also from multipolar neurones of Layer III. Pure IPSPs were obtained from ganglionic type neurons having their bodies located in Layer III. Radial cells have apical and basal dendrites. The apical dendrites of EPSP-IPSP cells terminate or give off prominent laterally spreading branches within sublayer IIe, f while those of EPSP cells distribute more superficially in sublayers IIa-d. Autoradiographic studies (Hunt, S.P. and Kunzle, H., J. Comp. Neurol., 15:173, 1976) have shown the presence of 3H-GABA accumulating axon collaterals in sublayer IIf. Our data suggest that EPSP-IPSP cells may receive their inhibitory input by way of such GABA related system.

237.12 THE ANURAN NUCLEUS PRETECTALIS II: LAMINAR ORGANIZATION OF AFFERENT TERMINALS. Neil M. Montgomery & Katherine V. Fite, University of Massachusetts, Amherst, Massachusetts 01003

The anuran nucleus pretectalis (nPt) receives afferents from the central retina and is substantially involved in the neural circuitry underlying optokinetic nystagmus (Montgomery, et al., 1982a,b). The geometry of afferent terminations in nPt was investigated using both HRP and autoradiographic techniques.

Bilateral optic nerve transections soaked with HRP saturated gelfoam showed dense labelling of optic terminals throughout nPt. With unilateral optic nerve soaks however, the contralateral nPt showed a restricted dense-core of HRP label in the superficial portion of the nucleus with sparcer HRP label occurring in the surround. Ipsilateral retinal afferents arborize throughout nPt except the dense-core region. Thus a central contralateral-core, ipsilateral-surround configuration exists for retinal afferents.

Afferents from the accessory optic system (AOS) arborize only in the dense-core region, following restricted HRP injections of nBOR. Afferents from the nucleus of the posterior commissure arborize in all parts of nPt except the dense-core, while afferents from the tectum and anterior thalamus appeared to arborize throughout the nucleus without discernible pattern.

This lamination of afferent terminals in nPt was correlated with cytoarchitectural material in which the somata of the large neurons of the nucleus cluster around the dense-core region. This specific population of large cells should thus receive inputs from both the contralateral retina and AOS near their somata and afferents from the nucleus of the posterior commissure along their outer dendritic extensions.

Similar laminated projections to the pretectal nucleus of the optic tract have been reported from the retina in opossum (Linden & Rocha-Miranda, 1981) and from the AOS in rat (Blanks et al., 1982).

Supported by NSF-8012350.

237.13 FUNCTIONAL ANALYSIS OF LMmc AND nBOR OF PIGEON ACCESSORY OPTIC SYSTEM. B. Morgan*, B. J. Frost, P. Ramm*, and J. Chown*. Dept. of Psychology, Queen's University, Kingston, Ontario, Canada. K7L 3N6

We have used unit recordings and ^{14}C-2-Deoxyglucose (^{14}C-DG) autoradiography to specify responses of the pigeon accessory optic system (AOS) to whole field visual stimulation. The AOS is a visual pathway processing information required for control of stabilizing eye movements during motion of the entire visual array. It includes the nucleus of the basal optic root (nBOR), and, possibly, the nucleus lentiformis mesencephali, pars magnocellularis (LMmc). Cells in nBOR respond preferentially to large stimuli moving slowly in an upward, or downward and anterior direction. We now report that cells in LMmc respond preferentially to horizontal motion.

Pigeons were monocularly exposed to stimuli consisting of a large random visual noise pattern, moving slowly (2-4 deg/sec) in a horizontal direction. Single unit responses were recorded from the LMmc of 34 birds. Other birds (n=14) received injections of ^{14}C-2-DG while viewing the random noise pattern moved in either a horizontal or vertical direction.

Responses of LMmc cells were essentially similar to those of nBOR cells, except in their preferred stimulus direction. Both LMmc and nBOR cells responded optimally to very large, slowly moving random noise patterns, were spontaneously active, and showed no adaptation to continued stimulation. However, LMmc cells preferred temporal to nasal horizontal motion, while nBOR cells preferred upward, or downward and anterior movement.

Autoradiographic optic densities (OD), were read from components of the AOS. Ratios were constructed comparing ODs in structures ipsilateral to the exposed eye, to those contralateral to the exposed eye. Ratios <1 indicated increased functional activity in the contralateral structure. The LMmc was selectively activated by horizontal movement (mean I/C=0.78), and nBOR by vertical movement (mean I/C=0.84).

The autoradiographic and unit data converge to suggest that LMmc processes horizontal whole-field motion, and nBOR processes vertical whole-field motion. Thus LMmc and nBOR, components of the AOS, are well adapted to detect retinal slip produced by self-induced motion in three planes.

This research was supported by NSERC grant A0353 and MRC grant MA7244 to B.J.F.

237.14 ULTRASTRUCTURAL CHARACTERIZATION OF A PRETECTAL NUCLEUS, THE LENTIFORM NUCLEUS OF THE MESENCEPHALON (LM). M.D. Gottlieb and O.C. McKenna. Biol. Dept., City College of CUNY, New York, N. Y. 10031.

The avian LM is a pretectal retinorecipient nucleus that is considered to be homologous to the nucleus of the optic tract (NOT) in mammals. Both the LM and NOT have been shown to respond preferentially to slow horizontal movement of the visual world and are thought to signal horizontal optokinetic eye movements.

We examined the fine structure of the LM in four week old chicks using routine methods for electron microscopy. The LM contains two types of neurons that differ in size, one approximately 15 um x 25 um and the other approximately 15 um x 15 um. The somatic profiles of both have a pyramidal shape, evidence a smooth nuclear envelope, and are filled with cytoplasmic organelles, in cluding abundant rough endoplasmic reticulum. These neurons receive axosomatic input from several types of synapses which can be distinguished from one another on the basis of the shape of the synaptic vesicles found in the presynaptic terminals; they contain either round, flat, or a mixture of round and flat vesicles. In all types of axosomatic synapses rough endoplasmic reticulum or cisterna is commonly found subadjacent to the postsynaptic membrane. In the neuropil two types of axodendritic synapses are found. By far the most frequently encountered type contains large round vesicles 40-50 nm in diameter which are clustered at the presynaptic membrane adjacent to the synaptic cleft. The cytoplasmic surface of the postsynaptic membrane displays a marked density. The area of the presynaptic profiles varies; the larger ones usually make several synapses onto one or more dendrites while several of the smaller ones may make individual synapses onto a single dendrite. The presynaptic profile of the second type of axodendritic synapse, which is less frequently seen, contains a mixture of small flat and round vesicles, often clumped near the presynaptic membrane. These profiles participate in axodendritic synapses but as a rule do not make multiple synapses. Unlike the first type of synapse, these do not have a marked postsynaptic density.

In order to identify terminals of retinal origin, HRP-WGA was injected intravitreally and the LM reacted with DAB. HRP reaction product is seen in presynaptic profiles containing large round vesicles indicating that these terminals are of retinal origin. In addition, label is seen in postsynaptic profiles suggesting that transsynaptic transport has occurred.

This study indicates that the most frequently encountered axonal terminal is retinal in origin. The presence of other axonal elements implies that retinal information may be modified at the level of the LM. We are currently investigating the origin of these other terminals. (Supported by NIH EY 03613)

237.15 VISUAL DEPRIVATION DISTURBS POSTNATAL DEVELOPMENT OF THE ACCESSORY OPTIC SYSTEM AND OPTOKINETIC BEHAVIOR. Olivia C. McKenna, Jose Velez*, Bradford Taylor* and Josh Wallman. Dept. of Biology, City College of the City Univ. of N.Y., New York, N.Y. 10031

Retinal slip signals, which provide the input for optokinetic responses, are largely transmitted through the accessory optic system. In birds, the principal nucleus of this system is the nucleus of the basal optic root (nBOR). A previous metabolic mapping study using 2-deoxyglucose (2DG) in 3 week or older chicks has shown that upward movement of the visual world results in labeling in the dorsal portion of the nBOR (nBORd) whereas downward and anterior movement of the visual world results in labeling in the nBOR proper. In chicks less than 1 week old this functional separation is not seen; instead, both divisions of the nBOR are equally labeled whether upward or downward motion is used as the visual stimulus. Furthermore, when optokinetic nystagmus (OKN) is measured in non-horizontal (vertical, torsional and intermediate) directions, the best OKN gain is different in hatchlings than in older birds. Together these results suggest that a postnatal reorganization of the nBOR occurs which, in turn, affects optokinetic eye movements. In the present study we tested whether lack of visual experience during the first three postnatal weeks influences the development of this system.

At hatching both eyes of chicks were covered with translucent occluders which were kept in place for 3 weeks. For the metabolic mapping studies animals were injected with ^{14}C-2DG and positioned in a rotating drum with both eyes open so that one eye viewed upward motion and the other eye viewed downward motion; since the visual pathways in chicks are essentially crossed, differential stimulation of the two eyes results in differential labeling of the two sides of the brain. Autoradiograms of the brains did not show the functional separation of the nBOR usually seen in 3 week old chicks; instead, the label was uniformly distributed within the nBORd and nBOR proper whether the corresponding eye viewed upward or downward motion. For the OKN experiments eye movements of animals were measured in a variety of directions. Preliminary findings show that visual deprivation impairs OKN in non-horizontal stimulus directions much more severely than in horizontal directions.

Together these results suggest that postnatal reorganization of the nBOR and the consequent optokinetic response system require visual experience to develop normally.
(Supported by NIH EY 03613 and EY 2937)

VISUAL CORTEX: INTRINSIC ORGANIZATION III

238.1 CONVERGENCE ON NEURONS IN LAYER IV (CAT AREA 17) OF LATERAL GENICULATE TERMINALS CONTAINING ROUND OR PLEOMORPHIC VESICLES. G.Einstein, T.L. Davis, and P. Sterling. Dept. of Anat., Univ. of Pennsylvania, Phila., PA 19104.

We observed that terminals from the lateral geniculate nucleus (LGN) labeled by anterograde transport of radioactive amino acids are of two morphological types: one contains round vesicles and forms an asymmetric synaptic contact; the other contains pleomorphic vesicles and forms a symmetric synaptic contact(ARVO, 1983). The LGN terminals with round vesicles are distributed throughout the depth of layer IV; those with pleomorphic vesicles are distributed in IVc and deep IVab. We believe that the terminals with pleomorphic vesicles arise from the small LGN neurons (Guillery type III) that accumulate GABA and stain with antibody to GAD. Some of these apparently GABA-ergic neurons have been shown physiologically to be X-cells and to project to area 17. Cortical neurons in IVc and deep IVab might therefore receive convergent input from excitatory (Guillery type II) X-cells and inhibitory (Guillery type III) X-cells . We examined neurons in this region for evidence of such convergence.

Six neurons were partially reconstructed from electron micrographs of 138 serial autoradiograms in which LGN terminals had been labeled by transport of 3H-proline and leucine. Consistent with the observations of others, LGN terminals with round vesicles were presynaptic to dendrites, somas, and spines; LGN terminals with pleomorphic vesicles were presynaptic to dendrites and somas but not to spines. Five of the reconstructed neurons were in IVc. They had medium somas (13-15μ) with pale cytoplasm, and each contained a cytoplasmic laminated body (CLB). Synaptic contacts were distributed to the somas at a density of 19-33/100μm^2. Most somatic contacts were made by terminals that contained pleomorphic vesicles, their ratio to those with round vesicles (F/R) ranging from 4-7. LGN input to the soma and proximal dendrites of these cells was sparse, representing only about 3% of all contacts; however, both types of LGN terminal were represented on each neuron. One reconstructed neuron was from the border of IVab/IVc. It had a medium soma (15μ) with dark cytoplasm and a folded nucleus. Synaptic contacts were distributed to its soma at a density of 34/100$\mu m^2$and LGN input to the soma was heavy, representing 21% of all contacts. LGN terminals of both types were represented roughly equally on the soma (13, pleomorphic; 15, round). In summary, of the six cortical neurons studied, all received LGN input. Whether the distribution from the LGN to these neurons was sparse or dense, both types of terminal were about equally represented. This suggests that the receptive field properties of cortical neurons deep in layer IVab and in IVc may be influenced by inhibitory input as well as excitatory input from X-cells in the LGN. Supported by NEI EY00828.

238.2 THE ORGANIZATION OF MACAQUE GENICULOSTRIATE TERMINALS VISUALIZED AFTER INTRAVITREAL INJECTION AND THE TRANSNEURONAL TRANSPORT OF WGA-HRP. S.K. Itaya, P.W. Itaya* and G.W.Van Hoesen. Depts. of Anatomy and Neurology, Univ. of Iowa, Iowa City, IA 52242.

In previous research we demonstrated that wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) can be used as a transneuronal marker of visual pathways (Itaya & Van Hoesen, 1982). To study monkey geniculostriate terminals, we made intravitreal injections of WGA-HRP, then processed coronal brain sections for TMB histochemistry (Mesulam, 1978). All labeling in visual cortex was terminal labeling; no WGA-HRP labeled cell bodies were observed, and few labeled intracortical axons were seen. Cortical layers in area 17 were numbered according to Lund (1973). A small amount of reaction product was observed in layer I, mainly in the calcarine fissure and usually above labeled patches in II and III. Lightly labeled patches occurred in II and III throughout the striate cortex, usually over ocular dominance columns in IVC. In IVA, terminal labeling in tangential sections clearly showed a honeycomb pattern in patches which usually corresponded to ocular dominance columns in IVC. In IVC there was a clear pattern of ocular dominance column labeling; additionally, intercolumnar labeling was also obvious. The ocular dominance columns themselves showed a range of shapes and sizes. Generally, IVCβ was more heavily labeled than IVCα, but in a small number of instances, this pattern was reversed. We also found examples where labeling was continuous between ocular dominance columns, either in IVCα, IVCβ, or in a thin layer comprising the lower one-third of IVCβ. Layer VI labeling was patchy, and usually coincident with ocular dominance columns in IVC. When bilateral injections of WGA-HRP were compared with semiserial sections stained for cytochrome oxidase (Wong-Riley, 1979), the labeling patterns from both techniques were similar with patches in II and III, honeycombs in IVA, a band in IVC, and patches in VI.

Several conclusions may be drawn from our results. 1) WGA-HRP is transported only in an anterograde-anterograde direction; all of our evidence to date also supports the hypothesis that WGA-HRP is transsynaptically transported in the visual system. 2) The transneuronal WGA-HRP technique confirms the known anatomy of geniculostriate terminals but, in addition, suggests the following: 3) Left and right retinogeniculostriate terminals are not completely segregated in the ocular dominance columns. 4) Retinogeniculostriate input occurs in patches in II and III. 5) The labeling of retinogeniculostriate terminals in IVC suggests a suborganization within the ocular dominance slabs. 6) Cytochrome oxidase staining coincides with geniculostriate terminals. (Supported in part by NS 14944 to G.W.V.H.).

238.3 PYRAMIDAL AND STELLATE NEURONS IN MONKEY VISUAL CORTEX LABEL FOR DIFFERENT PEPTIDES. Anita Hendrickson. Dept. Ophthalmology RJ10, University of Washington, Seattle WA 98195.

The peptides somatostatin (SOM), alpha melanocyte stimulating hormone (aMSH) and avian pancreatic polypeptide (APP) have been localized in Macaca monkey visual cortex using immunocytochemical techniques at the light and electron microscopic level. Animals were perfused with 4% paraformaldehyde containing 0.34% lysine and 0.005% periodate; some animals had received intracortical injections of 15ug/ul colchicine 24hrs before sacrifice. For LM frozen sections were reacted using standard methods for the peroxidase-antiperoxidase method of Sternberger. For EM the same method was used for vibratome sections, only the times in antisera and amount of Triton was reduced to provide better tissue preservation.

The two peptides SOM and APP labeled similar cell populations, but with different intensities. The most common cell was found in the white matter just under layer 6, and was seen throughout the occipital pole with no difference noted among visual areas. These horizontally oriented neurons have curved dendrites which send rising branches up into layer 6 and above. A single straight process, presumably the axon, arises from the cell body or primary dendrite and travels upward through all layers, finally ending in an extensive tuft in layer 1. SOM or APP neurons are less common within visual cortex; most are stellate neurons with multiple dendrites and a complex, beaded locally-ramifying axon. They occur most often in layers 2 and 3 in both primary and secondary visual cortex. APP processes form an extensive network in layers 3, 4B, and 5A, but are almost absent in 4C of striate. SOM neurons are more difficult to stain than APP and may be less frequent.

aMSH labels mainly pyramidal neurons; preabsorption of diluted antiserum with 10uM aMSH completely abolishes this staining. Without colchicine the large pyramids of layers 5 and 6 in striate and layer 3 of prestriate are heavily labeled. With colchicine all pyramids label, especially the small cells in layers 2 and 3 which appear almost Golgi-like with dendritic spines and axon delineated. Bundles of axons in cortex and white matter are also labeled by aMSH. In all visual areas the granule cell layer of 4 contains very few stained cell bodies. At the EM level the immunocytochemical stain is found throughout the cytoplasm, especially around microtubules. Asymmetric synaptic terminals are sometimes stained as are all other regions of the pyramidal neuron.

The role for these peptides in visual processing must await neuropharmacological and neurophysiological testing.

Supported by EY-1208 and the Dolly Green Scholar Award of Research to Prevent Blindness, Inc.

238.4 THE FINE STRUCTURE AND INTRACORTICAL CONNECTIONS OF SMALL, LAYER IV, SPINOUS STELLATE NEURONS IN MONKEY STRIATE CORTEX. R. L. Saint Marie and A. Peters. Dept. of Anatomy, Boston Univ. Sch. of Med., Boston, MA 02118.

Several varieties of spinous stellate neuron have been described in layer IV of monkey primary visual cortex (Lund, J.S., J. Comp. Neurol., 147: 455-496, 1973). Of these, only the smallest have axons that routinely impregnate with the rapid Golgi and Golgi-Kopsch procedures. The fine structure and intracortical connections of these neurons are examined using the gold-toning EM procedure of Fairén et al. (J. Neurocytol., 6: 311-337, 1977).

Small, spinous stellate neurons have rounded cell bodies (7.5-10 um, diam.). The nucleus is also round, slightly eccentric and nearly fills the cell body. Rough ER is sparse and dispersed within the perikaryon with no well-developed Nissl bodies present. Few synapses occur on the cell body and these synapses are symmetric. As few as three primary dendrites may emerge from the cell and bifurcate 3-4 times. The resulting dendritic field is approximately spherical. Dendritic spines frequently terminate as a rounded head connected to the dendrite by a long thin stalk. Synapses on these spines are asymmetric almost exclusively whereas axo-dendritic synapses may be either symmetric or asymmetric.

The axons of small, spinous stellate neurons descend either from the cell body directly or, rarely, from the base of a primary dendrite. The initial segment possesses no prominent axo-axonal synapses. The descending axons of some small, spinous stellate neurons branch to form several recurrent collaterals which ascend vertically through the dendritic field as far as layer III. The recurrent axons of others ascend obliquely within layer IV. The axons of still other spinous stellates emit long, horizontal collaterals which terminate in a secondary plexus within layer IV. Synapses formed by the axons of all small, spinous stellate neurons are asymmetric and these synapses occur on dendritic spines for the most part, as well as on dendrite shafts. Light-microscopic examination of groups of well-impregnated neurons suggest that the axons of small, spinous stellate cells make synaptic contact with the dendrites of other spinous and non-spinous stellate neurons, with the apical dendrites of infragranular pyramids and their secondary branches, with the dendrites of layer III and IVA-B pyramidal neurons, and with the dendrites of the parent cell (autapses). Some of these synaptic contacts have been confirmed ultrastructurally.

Supported by NIH Grants NS07016 and T32NS07152.

238.5 THERE ARE INTERINDIVIDUAL DIFFERENCES IN THE NUMERICAL DENSITY OF FLAT-SYMMETRICAL (BUT NOT OF ROUND-ASYMMETRICAL) SYNAPSES IN AREA 17 OF CAT. C. Beaulieu* and M. Colonnier. Department of Anatomy, Laval University, Québec, Que. G1K 7P4.

The numerical density (Nv) of synapses has been estimated in individual laminae of the binocular and monocular regions of cat area 17, using a method of size-frequency distribution. Separate estimates were obtained for asymmetrical synapses with round vesicles (RA synapses) and for symmetrical synapses with flat vesicles (FS synapses). Five adult animals have been studied to date. For the total cortical thickness, the Nv of synapses was not significantly different for the two regions, being in the order of 180 million/mm^3 in the binocular and 175 million/mm^3 in the monocular region. Of these, approximately 84% were of the RA type and 12% were of the FS type (the remaining 4% could not be clearly classified as one or the other). There was no significant difference in Nv between binocular and monocular regions, for either of the two types. In contrast the total number of synapses under 1 mm^2 of cortical surface was 40% higher ($p < 0.005$) in the binocular region (310 million), than in the monocular region (225 million). This difference is due mainly to the greater thickness of the binocular region.

Although there was no significant difference between the number of RA and FS synapses per mm^3 of tissue in the binocular and monocular regions, the standard deviations in both regions were much higher for FS (~40% of the mean) than for RA (<10% of the mean) synapses. In an individual animal if the Nv of FS synapses was either high or low in the binocular region, it was correspondingly high or low in the monocular region. The animals, however, were very different from each other (as low as 13 million and as high as 38 million FS synapses/mm^3 of cortical tissue). For this reason, the two-way analysis of variance comparing the Nv of FS synapses in the binocular and monocular regions of the five animals which showed that there was no difference between the two regions for these animals, did demonstrate a significant difference between the animals ($p = 0.01$). This was not the case for the RA synapses, were the Nv was quite similar for both regions and for all animals. The interindividual differences for the FS synapses may be due to differences in age, breed or to environmental factors. In cortex, FS synapses are largely GABAergic and inhibitory (Riback '78, J. Neurocytol: 7,461). On the basis of the evidence presented by Leventhal and Hirsch ('80, J. Neurophysiol: 43,1111) that inhibitory connections may be particularly dependent on visual experience, we suggest that the interindividual differences are mainly due to the (unknown, and probably varied) rearing conditions of the animals used in the present study.

Supported by MRC grant MT3735.

238.6 ACTIVITY LABELLING OF THE VISUAL SYSTEM FOLLOWING INACTIVATION OF THE RETINAL "ON" CHANNEL WITH 2-AMINO-4-PHOSPHONOBUTYRIC ACID (APB). J.H. Sandell and P.H. Schiller*. Psychology Dept., M.I.T. Cambridge, MA 02139.

2-amino-4-phosphonobutyric acid (APB) selectively inactivates ON center bipolar cells when applied to the retina of the rhesus monkey (Schiller, 1982). As an adjunct to neurophysiological and behavioral studies using APB, we have begun to study the changes in the biochemical anatomy of the visual system which accompany chronic APB administration.

One rhesus monkey received six 0.075cc injections of 14mM APB into the vitreous of one eye (est. conc. = 525μM) over a 15 day period. The effectiveness of the APB was monitored by sampling LGN unit activity following the first and fourth injections and just prior to sacrifice. The ON response of LGN units in the injected eye laminae was abolished by this chronic injection procedure, although OFF responses remained plentiful. Serial sections from the striate cortex and the LGN were processed for AChE, BuChE and cytochrome oxidase activity, together with sections from a monkey which had been monocularly enucleated for 15 days.

The lateral geniculate nuclei from the APB treated animal contained normal AChE and BuChE activity and moderately decreased cytochrome oxidase activity in the injected eye laminae. As expected, the LGN of the enucleated animal had moderate reductions in AChE and BuChE activity in accordance with Graybiel and Ragsdale (1982), and dramatically decreased cytochrome oxidase activity in the deprived eye laminae.

The enucleated animal's striate cortex revealed the prominent AChE and BuChE stripes described by Graybiel and Ragsdale (1982). The cortex of the simultaneously processed APB treated animal showed no such stripes. By contrast, cytochrome oxidase stripes in layer 4c were well defined in the cortex of both animals, although those of the APB treated animal were somewhat fainter. The cytochrome oxidase patches above and below layer 4 were essentially normal in the APB treated animal; they did not have the obvious alternating rows of darkly and faintly labelled patches seen in the enucleated animal. These findings show that the changes in cortical activity produced by blocking the ON system for 15 days can produce clear modulation of cytochrome oxidase activity but not AChE or BuChE activity in the rhesus monkey. Supported by: BNS 8019714, EY 00676 and NIGMS 2 T32 GM 074-84.

Schiller, Nature, 297:580-583, 1982.
Graybiel and Ragsdale, Nature, 299:439-442,1982.

238.7 HOW IMPORTANT ARE INHIBITORY SUBREGIONS FOR THE VELOCITY UPPER CUT-OFF IN THE CAT VISUAL SYSTEM ? J. Duysens, G.A. Orban, H. Maes* and J. Cremieux*. Laboratorium voor Neuro- en Psychofysiologie, K.U.L., Campus Gasthuisberg, B-3000 Leuven (Belgium).

In theory, the absence of responses to a fast moving light bar could be related to interactions between excitatory and inhibitory regions in the visual field of geniculate or cortical neurons. This may be due to retro-active inhibition, elicited by the entry of the light bar into a short-latency inhibitory zone after crossing the discharge region (Goodwin and Henry, J. Physiol.(Lond.), 277, 467, 1978). Or it may be due to pre-active inhibition, in which the excitatory effects, caused by the crossing of the discharge zone, are neutralized by the lingering inhibitory effects caused by the preceding entry into an inhibitory region.

The contribution of these two mechanisms was explored in 94 cells (15 from LGN, 59 from area 17 and 20 from area 18) by testing the effect of masking of these inhibitory zones on velocity sensitivity. Cells which rely on inhibitory zones for their velocity upper cut-off were expected to have a higher cut-off when the inhibitory zones were masked.

In the LGN two X-ON cells were found to respond to much higher velocities when only the most sensitive part of the center was stimulated. The latency of the inhibition was not shorter than the latency of the excitation so that pre-active inhibition is the most likely explanation for the velocity upper cut-off of these cells.

At the level of the cortex, the most dramatic effect of limiting the exposed receptive field was an overall decrease in response level. Since such decrease was not apparent in the LGN material it follows that the need for spatial summation increases steeply at the cortical level. Cortical cells were divided into an ON and OFF group depending on the presence of dominant ON or OFF subregions. When ON cells were tested with a light bar and OFF cells with a dark bar it was found that most cells showed a slight shift of their velocity upper cut-off to lower velocities as a result of the mask induced reduction in response levels. In a few cases the cut-off shifted to higher velocities and evidence for retro-active inhibition was obtained for some of these cells. When OFF cells were tested with a light bar then shifts of the cut-off to higher velocities occurred more frequently and this seems largely due to the masking of pre-active inhibition.

It is concluded that inhibitory interactions between different parts of the RF play only a secondary role in the generation of a velocity upper cut-off in most cells of areas 17 and 18.

An alternative mechanism, based on the temporal integration characteristics of cortical cells, is likely to be of more importance in this respect (Duysens et al., Soc. Neurosci. Abstr., 8, 677, 1982).

238.8 CELLS ENCODING FOR RED, YELLOW, GREEN, AND BLUE IN LAYER 4 OF STRIATE CORTEX IN THE AWAKE MONKEY. R.G. Vautin* and B.M. Dow (SPON: C.J. Smith). Neurobiology Division, Physiology Department, School of Medicine, SUNY, Buffalo, NY 14226.

Complete color tuning curves have been obtained for 218 cells in foveal striate cortex of alert, behaving macaques. Each cell was tested with its optimal spatial stimulus. Test colors, provided by 14 interference filters and four gelatin filters (purple) matched for human photopic luminosity, were presented at luminance levels sufficient to evoke vigorous responding from individual cells. Data were plotted as percent maximal response, and a tuning index (1-the area under the curve) obtained for each cell.

Cross-correlations of 183 tuning curves having a tuning index of at least .25 revealed 132 which correlated at a level of .9 or better with at least one other tuning curve. Sixty-four tuning curves fell into the six largest cross-correlation groups, containing 15, 14, 14, 9, 6, and 6 cells. Mean tuning curve peaks for these six groups were at 450, 656, 656, 506, 506 and 577 nm, respectively. The more broadly tuned 656 and 506 nm groups were excluded from further analysis. Mean tuning curves for the other four groups suggest an opponent relationship between 656 and 506 nm cells (red vs. green) and between 577 and 450 nm cells (yellow vs. blue). Cone inputs to the four groups, estimated from Smith and Pokorny's human cone sensitivity functions (Boynton, Human Color Vision, 1979) by means of a curve-fitting procedure, also suggest opponent pairs, with cortical red and green cells receiving input from long vs. middle wavelength cones, and cortical yellow and blue cells receiving input from long plus middle vs. short wavelength cones.

Microdrive depth readings and histological lesion sites suggest that red, yellow, green, and blue cells are concentrated in layer 4 of striate cortex, whereas cells responding optimally to other colors (e.g., orange, cyan, purple) are located more typically in upper and lower layers. Combining the mean curves of red (r), yellow (y), green (g), and blue (b) cells according to Hurvich and Jameson's (J. Opt. Soc. Amer. 45:602-616, 1955) opponent hue model, gives a curve similar in most regards to those obtained from human observers by means of either hue cancellation or hue naming techniques (Werner and Wooten, Percept. Psychophys. 23:371-374, 1979). The red, yellow, green, and blue cells found in layer 4 of the monkey's striate cortex may therefore constitute the striate cortical correlate of the 4 primary colors of the Hering-Hurvich-Jameson opponent-colors theory.
(Supported by NIH grants EY02349 and T32 EY07019)

238.9 THE NEURONAL CODING OF ROTATING BAR STIMULI IN PRIMARY VISUAL CORTEX OF CAT. J.C. Pearson*, Dept. of Physics; D.M. Diamond*, Dept. of Psychobiology; T.M. McKenna, Dept. of Psychobiology; P.C. Rinaldi, Dept. of Neurosurgery; G.L. Shaw, Dept. of Physics; N.M. Weinberger, Dept. of Psychobiology, Univ. of Calif. Irvine, Irvine, Calif. 92717

Investigations of the functional organization of area 17 have revealed that cells having a common optimal bar orientation are arranged in minicolumns. These studies usually have employed stimuli whose orientations were fixed during a given presentation. However, such simple "isolated" stimuli may not have revealed the full extent of the information processing capacity of visual cortical cells. This issue has been addressed by Shaw, et. al., (Exp. Neurol., 77:324, 1982) in a proposal to investigate dynamic interactions between minicolumns. Specifically, it was suggested that the responses to rotating bar stimuli would involve interactions between minicolumns, and reflect additional processing capacities.

Single unit activity was recorded from chronically prepared cats under neuromuscular blockade to insure constancy at the periphery. The stimuli consisted of a bar of light, (approximately 3° by 0.25°) "rotating" about its center, which is a fixed point in the visual field. The "rotation" consists of sequential presentation of the bar at 4-8 equally spaced angles. This sequential stimulation is predicted to involve interactions between minicolumns within a given ocular dominance column, hence, the response to a bar of a given orientation should depend on the sequence it is in. We have investigated dynamic stimulus parameters evoking neuronal discharge patterns which can not be explained simply as a composite of the responses to the "isolated" stimuli. Previously, we have reported such complex sequence-dependent responses in multiple-unit recordings (Shaw, et. al., Exp. Neurol.,79:293,1983). We have extended this inquiry to the discharges of single neurons and have found three sequence-dependent effects to date: (1) a significant difference between responses to a given orientation of a bar as a function of the direction of rotation, i.e., clockwise vs. counter-clockwise; (2) differences in response to identical sequential stimuli presented at slightly differing rates (1.0 Hz vs. 1.2 Hz.) -- there were no differences in response to identical isolated single bars presented at these same rates; (3) replacing one orientation in a clockwise sequence by a blank (no bar) of equal duration resulted in no change in the response to the next orientation -- in contrast, the responses to the other orientations were doubled. Such single neuron response properties appear to reflect network dynamics.

Supported by a U.C.I. FRP grant

238.10 ORIENTATION AND SPATIAL FREQUENCY DISCRIMINATION: CAT SINGLE CELLS AND HUMAN PSYCHOPHYSICS B.C. Skottun*, A. Bradley* and I. Ohzawa* (SPON: T. Cohn) School of Optometry, University of California, Berkeley, CA 94720

The spatial selectivity of single neurons in the visual cortex suggests an important role for these units in mediating spatial vision. Quantitative comparisons between behaviorally determined properties of spatial vision and response characteristics of single cells must incorporate not only the selectivity but also the variability of the neuronal responses. In this study we compare the performance limits of single cells in the cat's visual cortex (area 17) with the psychophysically determined orientation and spatial frequency discrimination limits of human observers, using techniques that take both factors into account while requiring few assumptions to be made.

All experiments were performed with sinusoidal luminance gratings. Human psychometric functions were obtained with a temporal-two-alternative-forced-choice method of constant stimuli. "Neurometric" functions for individual cells plot the probability of an increase in firing rate for stimulus changes of different magnitudes. The probabilities were estimated, using Receiver Operator Characteristic (ROC) analysis, by comparing firing rate distributions from two conditions out of a series of orientations (or spatial frequencies) that were presented in a randomly interleaved sequence.

For both single units and human observers a 75 % threshold criterion was adopted. The mean thresholds for 6 human observers were 0.56 deg and 3.6 % for orientation and spatial frequency, respectively. For the single neurons large inter-unit variability was evident with thresholds ranging from under 2 to more than 16 deg, and from about 4 to 48 % for orientation and spatial frequency, respectively. In our sample (N= 15 for orientation and N= 11 for spatial frequency) the "best cells" had orientation and spatial frequency discrimination thresholds of 1.85 deg and 3.95 %, respectively.

The similarity in resolution of single cells and human observers suggests that the information contained in the firing rate of individual neurons can be close to the level required to attain the performance measured behaviorally.
(We are indebted to G. Solar for preparing the cats for recording.)

238.11 A SEARCH FOR FUNCTIONAL BINOCULARITY IN THE PIGEON. B. J. Frost, M. A. Goodale, and J. D. Pettigrew. National Vision Research Institute, Carlton, Victoria, Australia 3053.

Although there is behavioral evidence to suggest that the pigeon uses stereopsis to make depth judgements, a neural substrate for stereoscopic vision has not been demonstrated in this species. One candidate for the necessary binocular integration is the visual Wulst which receives both ipsilateral and contralateral projections from the optic nuclei of the thalamus. In the owl, units have been recorded in the Wulst that are well-tuned for different retinal disparities, and accumulating anatomical and electrophysiological evidence suggests that the thalamic projections to the avian Wulst may be a functional analogue of the geniculostriate system in the mammal.

The present study was designed (1) to determine whether functional binocular interaction exists at the single-unit level in the pigeon's visual Wulst, and (2) to measure the amount of functional binocular overlap by combining eye position recording with visual field measurements derived from electrophysiology and ophthalmoscopy.

Electrophysiological recordings were made with glass-coated tungsten microelectrodes under Ketalar/Rompun anesthesia. Retinal landmarks (the pecten and ora terminalis) of each eye were plotted with a reversible ophthalmoscope. The position of the eyes was measured in alert birds with subconjunctival search coils. Single-unit recordings in the pigeon's Wulst showed two distinct retinotopically organized maps of visual space, one lying subjacent to the other in the hyperstriatum accessorium. A much larger proportion of the map was devoted to that part of the visual field immediately in front of the beak, and most cells with receptive fields in this area responded best to stimuli presented within 10 to 15 cm from the eye (in contrast, to units in the nucleus rotundus which responded to stimuli more than 200 cm from the eye). Despite the over-representation of the field in front of the beak, only a few binocular cells were encountered. Measurements derived from the eye position recordings and the retinal landmark plots indicated that when the pigeon's eyes were in the primary position (at rest and under anesthesia), the area centralis of each eye coincided with the ora terminalis of the other providing about 22^{o} of binocular overlap. Eye movements occurred in the alert bird, however, which were up to 20^{o} convergent of the primary position so that the areae centrales lined up and binocular overlap doubled to over 40^{o}. The failure to find many binocular units in the Wulst may therefore be due, either to the fact that monocular processing is favoured at times of divergent oculomotor activity, or to the technical difficulties associated with stimulating binocular units when the receptive fields would be divergent by some 20^{o}.

238.12 ANALYSIS OF INTRINSIC CONNECTIVITY IN CAT VISUAL CORTEX USING INTRACELLULAR DYE INJECTIONS IN VITRO. L. C. Katz* (SPON: A. J. Hudspeth). Div. of Biology 216-76, Caltech, Pasadena, CA 91125.

Intracellular, in vivo injection of horseradish peroxidase into neurons in cat area 17 has illustrated the previously unsuspected complexity of the intrinsic axonal arborizations of these cells (Gilbert, C. D., Weisel, T. N., Nature 280:120, 1982). However, since obtaining well filled cells, or cells in specific laminae is technically difficult in vivo, an in vitro brain slice preparation of cat area 17 has been devised. Intracellular staining of cortical neurons in vitro reveals detailed axonal arborizations whose extent and appearance are similar to those described in vivo.

350 µm slices were cut from the visual cortex of Nembutal-anesthetized adult cats, maintained in vitro with standard mammalian brain slice techniques, and intracellularly injected using lucifer yellow. Laminar borders were clearly visible. In experiments on six cats, 150 cells (mostly pyramidal) were intracellularly injected in layers II/III. The pyramidal cells had a stereotyped pattern of arborization. Collaterals which emerged from the cell body in the vicinity of the basal dendrites ascended and branched within the upper reaches of layer II and within layer I. Within layer I, the terminals often formed discrete clusters, as described in vivo. A number of long horizontal collaterals (extending over 1 mm) emerged from the main axon at the III/IV border. Often these collaterals sent vertically ascending branches back into layer II/III. No collateral branching was seen in layer IV. Another profuse collateral arborization (also extending for up to 1 mm) was observed within layer V. Smaller branches were observed in layer VI.

The electrical excitability of neurons in slices was apparently normal. Action potentials of filled cells averaged 50 mV amplitude and less than 1 msec duration at half height. Spontaneous EPSP's and IPSP's were frequently observed. Intracellular penetrations remained stable for over an hour, and the slices were viable for up to 12 hours.

I have used this preparation to determine whether the efferent connectivity of area 17 cells correlated with a specific pattern of intrinsic axonal arborization. A new fluorescent retrograde tracer (latex microspheres) was injected into the corpus callosum in vivo followed by preparation of brain slices and intracellular lucifer yellow injections in vitro. At least one double labelled cell at the lamina III/IV border was clearly a spiny stellate cell with some axon collaterals running horizontally along the III/IV border and with others ascending into lamina III. This confirms the observation (Innocenti, G. M., Fiore, L., Neurosci. Lett. 2:245, 1976) that some stellate cells can make long distance efferent connections.

238.PO **A MODEL OF A CORTICAL AREA BASED ON A GENERALIZED MORPHOLOGY OF THE NEOCORTEX.** W. von Seelen*, G. Krone*. Institut für Zoologie, Saarstr. 21, D-6500,Mainz, FRG. (SPON: K. Behrend)

The visual cortex of the cat comprises at least 13 areas each with a topographical representation of the visual field. One method of studying such a system is to lesion one area after the other and compare the performances of the animals in specific behavioral tasks. The outcome of such experiments has shown that there is a considerable degree of functional interaction between the areas.

To investigate principles of interaction we constructed a model of an area based on a generalized morphology. The model comprises six layers of pyramidal and stellate cells. Neurons are described by their dendritic and axonal density which is synonymous with afferent and efferent connectivity. As a rule, the afferent area of pyramidal cells increases from layer II to layer VI. Input to layer I is considered to be of cortical origin, input to layer to be of thalamic origin with no restrictions with no restriction to quality.

As a first step, we simulated the response of neurons in layers II to VI to an input to either layer I or IV, or to both of them. Despite the rigid and uniform design of elements and connections, the response profiles in space and time of the different layers show a surprizing variety which includes those of the known neurophysiological cell types from "simple" to "hypercomplex". The second step will be to simulate two such model areas and to investigate the effects of their interaction.

239.1 PHYSIOLOGICAL DISTRIBUTION OF PIRACETAM IN RATS. M.T. Tacconi* and R.J. Wurtman (SPON: A.L. McCall). Laboratory of Neuroendocrine Regulation, MIT, Cambridge, MA, 02139, USA.

Although previous studies suggest that levels of piracetam (2-oxo-1-pyrrolidine acetamide, Nootropil R , the prototype of nootropic drugs, are higher in brain (especially rat cortex), than in blood several hours after its administration, few data are available on its overall physiological disposition in experimental animals. We examined the distribution and fate of piracetam in rats given 100 or 1000 mg/kg of the compound by gavage, with or without the addition of ^{3}H piracetam as a tracer. Peak levels in serum were obtained after about 60 minutes; thereafter levels decayed following a two exponential curve, with a short (2 hours) and a longer (3.5 hours) half life. Brain levels equaled those of serum at about 4 hours, after which brain levels fell exponentially but more slowly, so that the drug's concentration was about 2 fold higher in brain than in serum. No evidence could be obtained for significant metabolism of the drug, either after its oral administration or when it was incubated with liver homogenates. Brainstem piracetam levels after a single dose were lower (by 30-40%) than those in olfactory bulb, superior colliculi or cortex. After its oral administration piracetam accumulated in the cytosol of subcellular preparations of liver and brain, showing low (2-3%) binding to particulate organelles. Repeated doses (100 mg/kg, 7 days) of piracetam failed to elevate brain levels beyond those observed after single doses. These data suggest that piracetam probably distributes within two metabolic compartments, a central compartment, including the plasma and easily-diffusible tissues, with a high elimination rate; and a compartment with a lower diffusion rate. Since piracetam has a Chloroform/water partition coefficient of 0.04, the drug might meet some resistance in crossing the blood brain barrier in both directions so that its clearance from the brain is retarded in comparison to that of the serum.

239.2 MEMORY PROTECTIVE AND ENHANCING EFFECTS OF ANIRACETAM (Ro 13-5057) IN MICE TESTED IN AVOIDANCE PROCEDURES. G. Vincent*, A. Verderese*, and E. Gamzu (SPON: L.M. Bartoshuk). Dept. Pharmacology I, Hoffmann-La Roche Inc., Nutley, NJ 07110.

Previous reports from our laboratories have shown that the nootropic agent aniracetam, a piracetam analog (1-anisoyl-2-pyrrolidinone), protected against a non-convulsant electrobrain shock (EBS)-induced disruption of retrieval in a one-way shuttle avoidance procedure (Gamzu et al., Soc Neurosci Abstr 7:525, 1981; Cumin et al., Psychopharm 78:104-111 1982).

We have developed an automated platform-jump avoidance procedure using mice, which allowed for manipulation of acquisition and retention of the avoidance behavior. Mice are trained on Day 1 to avoid a grid shock by jumping onto a drop platform. This training consisted of 2 separate 5-trial sessions spaced 4 hours apart. Testing occurred 24 hours later using a 10-trial session.

In aniracetam pretreated mice (at 10, 30, and 100 mg/kg) EBS applied prior to testing did not disrupt the avoidance response. Thus, aniracetam was protective against memory retrieval corroborating our earlier observations in the shuttle avoidance. Low avoidance behavior can be produced by eliminating the second training session. In this test, aniracetam up to 100 mg/kg, administered immediately after a single training session, failed to effect memory consolidation in normal mice. This result suggests that normal mice may be responding close to an optimal level in this test. Consequently the next two experiments focused on the poor learners - i.e. mice that failed to successfully escape the shock in the first training session. In these mice, aniracetam (100 and 300 mg/kg), given one hour before or immediately after the second training session, produced a significant improvement in avoidance performance compared to vehicle-treated animals. This post-training paradigm suggests that part of the performance can be attributed to improved consolidation of memory. Our findings confirm the memory-protective effects of aniracetam. Furthermore, experimental evaluation of nootropic drugs might be expanded to include situations involving experimentally impaired learning and memory or animals with learning/memory deficits.

239.3 A RAPIDLY ACQUIRED, APPETITIVELY MOTIVATED, SERIAL SPATIAL DISCRMINIATION REVERSAL IN RATS FOR EVALUATING MANIPULATIONS OF LEARNING AND MEMORY. E. Gamzu, E. Boff*, M. Zolcinski*, G. Vincent*, and T. Verderese*, Pharmacology I, Hoffmann-La Roche Inc., Nutley, NJ 07110

Animal tests for evaluating manipulations of learning and memory range from simple one-trial procedures to complex learning situations requiring long periods of training. We have developed an automated procedure in which over 90% of naive rats can be trained in a single 1 hour session. A spontaneous food-seeking response to a feeding device is reinforced on a schedule that eventually requires 10 responses for each food pellet (FR10). 24 hours later the rats can obtain 5 reinforcements for 20 responses (i.e., an FR4) from one of two adjacent feeding devices. Responses to the second device are not rewarded and are counted as errors. This unsignalled spatial discrimination is then reversed 13 times in a single session (1 hour maximum). Errors and qualitative measures of performance are analyzed in each discrimination component. Control rats show the expected high error rate during the first few reversals followed by a gradual improvement in performance. Repeated exposure to such test sessions continue to improve performance for at least 8 sessions.

Eighteen compounds, chosen on the basis of clinical use for treating cognitive deficits, or efficacy in animal "learning" procedures were administered prior to a single test session. Only piracetam (1000 mg/kg p.o.) and diphenylhydantoin (10 mg/kg p.o.) significantly improved performance, but these effects could not be replicated.

Aniracetam (Ro 13-5057) and piracetam were administered daily prior to 4 successive test sessions. Aniracetam (10 and 30 mg/kg i.p.) significantly improved performance on the second test session. Piracetam had no effect on any measure.

This test has also been useful in demonstrating deficits and strain differences in learning. Rats with lesions of the magnocellular nuclei of the basal forebrain learned this task significantly more slowly than appropriate controls (see Lerer et al., this meeting). In addition, spontaneously hypertensive rats were superior to Wistar-Kyoto normotensive rats (Vincent et al., Fed. Proc. 1983, 42:1347). Finally, we demonstrated that mature (12-month-old) rats made significantly more errors than 51-day-old rats.

239.4 EFFECTS OF ANIRACETAM (RO 13-5057) AND DIPHENYLHYDANTOIN ON HIPPOCAMPAL AFTERDISCHARGE IN RATS. S.A. Stwertka, D.A. MacNeil, and E. Gamzu, Dept. of Pharmacology I, Hoffmann-La Roche Inc., Nutley, New Jersey 07110.

Transcorneally administered subconvulsant electrobrain shock (EBS) disrupts the memory retrieval of mice in an avoidance procedure. Protection against this EBS-induced disruption can be produced by a variety of pharmacological agents used to treat cognitive deficits in patients (Gamzu & Perrone, Soc. Neurosci. Abstr. 1981, 7:525). We now report that in mice with bipolar electrodes implanted in the hippocampus, those EBS parameters that lead to memory disruption in an avoidance procedure also produce a hippocampal afterdischarge (AD). Moreover, the current threshold for producing this AD correlates highly with the threshold necessary to produce a memory disruption. This suggests that the previously reported pharmacological protection might be the result of a direct change in hippocampal reactivity to the EBS.

To test this possibility, we determined the effects of aniracetam (Ro 13-5057), diphenylhydantoin (DPH), phenobarbital (PHE), and chloral hydrate (CH) on electrically-evoked hippocampal AD activity in Sprague-Dawley male rats with chronic bilaterally implanted electrodes in the dorsal hippocampi. Following one to two days recovery, the animals were paralyzed with Flaxedil (80 mg/kg i.p.) and placed on a respirator during stimulation and recording. Constant current stimulation consisted of 2 second trains of 80 Hz biphasic square pulses of 4 msec duration delivered to one of the electrodes. Thresholds to evoke an AD were established over a period of three consecutive ADs at 15-20 minute intervals at the same current intensity. All drugs were administered i.v. through a lateral tail vein and were tested for effects on AD threshold, duration, and spread (to the contralateral electrode). PHE and CH significantly raised the threshold for evoking an AD. In contrast, DPH (50 mg/kg i.v.) suppressed the duration and spread of the AD, but had no detectable effect on the AD threshold. Aniracetam (1-100 mg/kg i.v.) had no effect on the threshold, spread, or duration of the AD. Thus, while it is possible that the protective effect of DPH against EBS-induced memory disruption is related to its ability to suppress the spread of AD activity in the hippocampus, the memory protective effects of aniracetam are most reasonably attributed to some other as yet unknown mechanism of action.

239.5 HOE 175: ITS EFFECT ON DIFFERENT LEARNING TASKS IN MICE. F. J. Hock and J. L. McGaugh. HOECHST AG, D-6230 Frankfurt/M. 80, Germany and Department of Psychobiology, University of California, Irvine, CA 92717.

Hoe 175, 3,8-dimethyl-4-phenyl-5,6,7,8-tetrahydropyrazolo (3, 4-b) (1,5)diazepine-1H,4H-5,7-dione, is a new compound with marked effects for nootropic activity. Though it is a pyrazolodiazepine the compound produced a slight effect in classical anxiolytic tests. We investigated the effect of Hoe 175 on (1) acquisition and retention in an one-trial passive (inhibitory) avoidance task and on (2) retention of an active avoidance task in mice. The apparatus used for the experiments consisted of a light (L) and a dark (D) compartment, connected by a guillotine door.

(1) Passive (Inhibitory) Avoidance. During the acquisition trial an unavoidable footshock (1 mA, 1.0 sec) was delivered immediately after entering D, followed by a maximal electroconvulsive shock (15 mA, 0.2 sec). Retention (the latency for entering D) was tested 24 hrs later. Hoe 175 was administered orally 90 min prior to both acquisition and retention. At doses from 3.0 - 12.0 mg/kg Hoe 175-treated mice had retention latencies significantly higher than those of controls.

(2) Active Avoidance. Animals were trained (8 trials) on the first day to avoid a footshock in D by moving to L. Each trial consisted of 10 sec to avoid the footshock followed by 20 sec footshock (350 μA) after which there was a 20 sec intertrial interval in L. The retention test trial was run 24 hrs later under the same parameters. During training and testing the time for entering L and the avoidance numbers were measured. Hoe 175 was administered intraperitoneally immediately after the last trial on training day. In comparison with controls, Hoe 175 in the dose range of 1.0 - 25.0 mg/kg significantly increased avoidances on the retention test.

239.6 REVERSAL LEARNING OF A SPATIAL-MEMORY TASK IS FACILITATED BY MEDIAL SEPTAL INJECTIONS OF 6-HYDROXY-DOPAMINE. L.E. Harrell, T.S. Barlow*, M. Miller*, J. Haring and J.N. Davis. Department of Neurology, Univ. Alabama, Birmingham, AL and Departments of Medicine (Neurology) and Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Electrolytic lesions of the medial septal region result in depletion of some hippocampal neurotransmitters including acetylcholine, norepinephrine (NE), serotonin, and Substance P. Behaviorally, medial septal lesions produce disruption of spatial and memory tasks. To determine whether a specific neurotransmitter might be responsible for dysfunction, we selectively depleted hippocampal NE and studied acquisition and performance of rats on a radial 8 arm maze.

Adult male Sprague-Dawley rats were trained to approach 4 baited arms and ignore 4 unbaited arms of an eight arm maze. Daily testing was performed and criterion of learning was defined as selection of 4 baited arms out of the first 5 arm choices on each of 5 consecutive days. Rats were assigned to either an acquisition (A) or performance (P) group. Group A rats were treated either with vehicle or 8 ug of 6-hydroxydopamine in the medial septal region prior to training, while rats in Group P were treated after mastery of the task. After attainment of either initial criterion (Group A) or after reaching a second criterion (Group P) all animals underwent a reversal procedure in which baited and unbaited arms were exchanged.

Treatment with 6-hydroxy-dopamine either prior to (Group A) or after mastering of the task (Group P) did not effect animal's ability to either learn or perform in this particular paradigm. Reversal performance was, however, significantly enhanced in all treated animals. These animals required approximately 25 trials to achieve criteria versus 40 trials in controls.

Our data suggest that hippocampal NE is not necessary for initially learning or maintenance of behavior in this particular spatial/memory task. Unexpectedly, we found that loss of hippocampal NE facilitated reversal learning. This finding may be important to understanding mechanisms of attention and interference since others have proposed a major role for the NE containing dorsal bundle in these behaviors.

239.7 AMPHETAMINE REVERSES LEARNING DEFICITS IN DOPAMINE DEPLETED RAT PUPS. R. S. Wool*, B. A. Shaywitz, M. H. Teicher, G. A. Anderson*, D. J. Cohen*, and D. A. Weldon. Dept. of Ped. and Neuro., Yale Univ. Sch. Med., New Haven, CT 06510 and Dept. of Psychology, Hamilton College, Clinton, NY 13323.

Depletion of brain dopamine in neonatal rats produces a constellation of changes in behavior that includes transient hyperactivity, deficits in habituation, and learning impairments in avoidance and escape tasks. We have recently found that dopamine depleted rat pups show deficits in appetitive learning performance as early as 8 days of age, and that these impairments can be reversed by treatment with the dopamine agonist apomorphine before conditioning (Weldon et al., Pharmac. Biochem. Behav. 1982, 17, 1281). The purpose of the present experiment was to determine whether administration of amphetamine would also produce a reversal of the performance deficits.

Selective depletions of brain dopamine were produced in 5 day old rat pups by administration of desmethylimipramine (20 mg/kg, i.p.) followed by intracisternal 6-hydroxydopamine (100 μg in 20 μL of saline). The next day each animal received an intratongue cannula while anesthetized with ether, and on day 7 each pup was conditioned by the presentation of licorice odor paired with the presentation of infant formula. Animals were tested the next day in a rectangular apparatus with a wire screen floor. Licorice extract was placed under one half of the floor, and learning performance was determined by measuring the percentage of a 5-min period that the animal spent over the side with the conditioned stimulus. Animals received injections of amphetamine (0.5 mg/kg, i.p.) prior to conditioning and testing.

In animals treated with saline before both conditioning and testing, control pups spent 85% of the time and depleted pups spent 40% of the time over the conditioned stimulus. Amphetamine administration prior to conditioning (but not testing) produced a significant improvement in the performance of depleted animals. In controls, amphetamine treatment prior to either conditioning or testing produced an impairment in performance. These results are consistent with our previous investigation of apomorphine effects in this task and support the hypothesis that dopamine plays an important role in learning in young animals.

239.8 RETENTION DEFICIT AFTER D-AMPHETAMINE TREATMENT: MEMORY DEFECT OR PERFORMANCE CHANGE? W. W. Beatty. Dept. of Psychology, North Dakota State Univ., Fargo, ND 58105.

Psychomotor stimulants such as amphetamine often facilitate long-term memory, either by enhancing consolidation or promoting retrieval. By contrast, these agents disrupt short-term memory (STM) in a variety of different tests. Kesner, et al. (1981) have interpreted these latter effects as support for the idea that arousal accelerates the loss of information from STM. But since amphetamine causes numerous changes in performance, alternative explanations of the deficit are also plausible. In an attempt to separate drug effects on memory from those on performance, the effects of d-amphetamine sulfate on spatial memory in the radial maze were studied in rats. The unusually long span of accurate STM in this setting permits drug administration within the retention interval as well as prior to the to-be-remembered event. (TBRE). In rats tested at a 5-hr retention interval (imposed between choices 4 and 5 in an 8 arm maze), 1 mg/kg d-amphetamine disrupted retention only when given 0.5 hr before the retention test. A higher dose (2 mg/kg) interfered with retention if given 0.5 hr before the TBRE or 0.5 hr before the retention test. Neither dose altered retention when given 0 or 2 hr after or 3 hr before the TBRE. At a 5 hr retention interval 3 mg/kg d-amphetamine impaired performance if given 2 hr after the TBRE, but not when given 0 hr after or 3 hr before the TBRE. However, when the retention interval was lengthened to 7 hr, administering 3 mg/kg d-amphetamine 2 hr after the TBRE did not degrade retention. The effects of d-amphetamine on spatial memory are best explained in terms of the well known effects of the drug on motor activity and appetite. Similar changes in performance may account for the "memory" impairments observed after amphetamine treatment in other tasks that measure STM.

239.9 EFFECTS OF PHYSOSTIGMINE ON MEMORY DEFICITS INDUCED BY PRETRAINING ELECTRICAL STIMULATION OF THE DORSAL HIPPOCAMPUS IN MICE. J. MICHEAU*, C. DESTRADE* and R. JAFFARD* (SPON: M. CAUDARELLA). Lab. Psychophysiologie, Univ. Bordeaux I, 33405 TALENCE FRANCE

Previous experiments based on mouse-strain comparisons have suggested that differences in long-term memory formation as well as facilitatory effects of posttraining hippocampal electrical stimulation on retention might be related to differences in hippocampal choline acetyltransferase activity (ChAT)(JAFFARD et al., Brain Res., 133 , 277-289, 1977). In subsequent experiments we have shown that pretraining electrical stimulation of the hippocampus led to a decrease of this enzymatic activity, with a latency of 6 hrs, in BALB/c mice and that this delayed decrease was paralleled by long-term memory deficits (JAFFARD et al., Physiol. Behav., 22 , 1093-1096, 1979). Consequently, it was suggested that a positive correlation exists between hippocampal ChAT activity at the time of training and the effectiveness of memory consolidation (JAFFARD and DESTRADE, in The Genetics of the Brain, I. LIEBLICH, Ed., 299-322, 1982). Though the activity of ChAT is an essential factor in acetylcholine synthesis, it is not clear if in physiological conditions the enzyme functions with saturating concentrations of substrate and thus if it can be considered a limiting factor for acetylcholine synthesis.

The aim of the present experiments was to test the validity of our hypothesis that presynaptic cholinergic activity may have a functional significance for memory formation. The results show that pretraining electrical stimulation of the dorsal hippocampus in BALB/c mice, which induces a decrease of about 40% of hippocampal ChAT activity at the time of learning, results in deficits in retention scores in two appetitive learning tasks (operant conditioning in the Skinner box or a spatial-memory task using a 4-hole board). In both behavioral tasks the intraventricular injection of 1 µg of physostigmine 20 min before the acquisition session reverses the disruptive effect of pretraining hippocampal stimulation.

Our results seem to indicate that the memory deficits induced by pretraining electrical stimulation of the hippocampus result from both a decrease of ChAT activity and a reduction of acetylcholine availability in the hippocampal formation.

239.10 EFFECTS OF SCOPOLAMINE ON RECOGNITION MEMORY IN MONKEYS AFTER IBOTENIC ACID INJECTIONS INTO THE NUCLEUS BASALIS OF MEYNERT. T. Aigner*, J. Aggleton, S. Mitchell, D. Price, M. DeLong & M. Mishkin. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20205 and Depts. of Neurology and Neuroscience, Johns Hopkins Medical School, Baltimore, MD 21205.

Alzheimer's disease (AD) is accompanied by degeneration of neuronal cell bodies in the nucleus basalis of Meynert (nbM), the major source of cholinergic projections to widespread areas of the neocortex. This abnormality has been suggested as one explanation for the memory impairment in AD. To test this hypothesis in the monkey, we trained three experimental (E) and two control (C) cynomolgus macaques on a delayed nonmatching-to-sample task with trial-unique objects in a Wisconsin General Testing Apparatus. The animals were required to distinguish a sample object, presented 10 sec earlier, from a novel object. The animals were given 20 trials each day until they reached a criterion of 90 correct choices in 100 trials. In the E monkeys, the region of the nbM was identified electrophysiologically and bilateral injections were made of ibotenic acid (IA), a neurotoxin previously shown to destroy neurons in this region. Two weeks later, behavioral testing was resumed. Two of the E monkeys relearned the task in the minimal number of trials (100), while the third required an additional 20 trials. The monkeys were then given a series of performance tests involving delays of 30, 60, and 120 sec and lists of 3, 5, and 10 objects to be remembered. Each condition was tested for 5 consecutive days. The E group showed no impairment on these tasks when compared to the C group (92.1% vs 93.5% correct, respectively). In order to challenge the damaged cholinergic system further, the task was made more difficult (2 lists of 20 items to be remembered per session), and a dose-response curve was determined for scopolamine hydrobromide (Scop) by injecting .3,1,3,5.6,10, or 17.8 mg/kg i.m. 20 min before the session. The drug session was preceded by at least one drug-free control session (no injection or saline). Each dose of Scop was tested twice in a nonsystematic order. Scop produced a dose-related decrease in the number of objects correctly remembered by both groups of monkeys. However, at each dose tested, the E group made more errors than the C group. Linear regression analysis was used to compare the relative potency of Scop in the two groups. The dose of Scop which reduced correct responses to 80% of control values was 5.0 mg/kg for the E group and 12.4 mg/kg for the C group.

Our data indicate that although monkeys can still perform a recognition memory task normally after IA injections into the nbM, their performance is more easily disrupted than that of normals when challenged with Scop. These results add support to the view that the nbM cholinergic system plays a role in memory.

239.11 BEHAVIORAL TOLERANCE TO SCOPOLAMINE IN THE RAT USING A WORKING MEMORY TASK. W.S. Messer, Jr.*, G.J. Thomas and W.P. Hoss. Center for Brain Research, University of Rochester School of Medicine, Rochester, New York 14642.

In order to examine further the known role of the cholinergic septo-hippocampal system in working memory, male hooded rats of the Long-Evans strain were trained to perform a delayed alternation task in a T-maze. After rats demonstrated proficiency (100% correct for three consecutive sessions), guide cannulae aimed at the CA3 region of each hippocampus were surgically implanted. After recovery from surgery, animals were retested, injecting physiological saline (0.5 µL) before each session until proficiency was attained again. Rats were then assigned to one of two groups, receiving 0.5 µL injections of either scopolamine hydrobromide (60 mg/mL) or saline bilaterally.

Whereas saline injections had no effect on performance, the initial dose of scopolamine caused a significant impairment of working memory. Usually this impairment was manifest as a reduction to chance in the number of correct choices. Occasionally, however, a rat would choose correctly but show a marked increase in choice time. Animals regained their ability to alternate within two days following the initial injection. A second injection of the same dose of scopolamine two days after recovery did not cause an impairment in either the percent of correct choices or choice time.

These experiments suggest that the hippocampus has a functional capacity to overcome the effects of muscarinic receptor blockade. A number of mechanisms could be responsible for the development of tolerance to scopolamine, including an increase in the sensitivity of muscarinic receptors. This possibility will be explored using autoradiographic localization of muscarinic receptors within the hippocampus.

This work was supported in part by NIH research grant DA 01851 and NIH training grant 5T32 GMO 7136-08.

239.12 NICOTINE TOLERANCE AND LEARNING IN MICE. S. J. Jackson, M. J. Marks* and A. C. Collins*. Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309.

The involvement of the cholinergic system in learning and memory is well documented. The prevailing attitude is that this phenomenon involves effects at the level of the receptor. If cholinergic receptors could be manipulated prior to a learning experience, evidence for direct involvement of that receptor in learning might be obtained.

It has been demonstrated that muscarinic receptors can be down-regulated by chronic treatment with anticholinesterases and up-regulated by chronic treatment with muscarinic antagonists. Conversely, nicotine, a cholinergic agonist, produces up-regulation of nicotinic receptors in response to chronic administration. Chronic exposure to these drugs also results in various behavioral and physiological changes in response to a challenge dose of the same drug.

C57BL and C3H mice either were injected with 2.0 mg/kg nicotine 3X/day for 3 days or were chronically infused for 3 days with 2.0 mg/kg/hr nicotine. Subsequent to chronic exposure, the mice were challenged either with saline or with 1.0 or 2.0 mg/kg nicotine and were trained daily for 4 consecutive days on a Rotarod or in a successive discrimination maze. The nicotinic receptors in the various groups were assayed to determine if changes in these receptors were correlated with changes in the rate of learning.

The results indicate that behaviorally tolerant C57BL mice, trained on the Rotarod, learn at a faster rate than do saline controls or naive subjects. This finding suggests the possibility of a direct role for nicotinic receptors in the learning process.

239.13 EFFECTS OF PHOSPHATIDYLSERINE ON MEMORY AND PSYCHOMOTOR PERFORMANCE IN AGED RATS. R.L.Dean[1], J.Corwin[2], D.L.Watkins[1], J.P. Rotrosen[2,3], and R.T.Bartus [1,3]. Med.Res.Div., American Cyanamid Co., Lederle Labs, Pearl River, NY, [2]Manhattan V.A. Med. Center, NY, NY, [3]NYU Med. Center, NY, NY.

One of the most serious neurobehavioral dysfunctions associated with old age is loss of memory. Biochemical, pharmacological and electrophysiological evidence suggests that dysfunctions in central cholinergic mechanisms may play a critical role in this impairment (Bartus, et al., Sci., 1982). This age-related impairment can be partially reversed with cholinomimetic agents. In addition to cognitive deficits, deleterious effects of age are also observed on tasks designed to measure psychomotor coordination and muscle strength. Recent research by Marshall and Berrios (Sci., 1979) and Joseph, et al. (Neurobiol.Aging, 1980) suggests that these dysfunctions might be reduced by pharmacologically compensating for age-related alterations in dopamine function.

Phosphatidylserine (PS), a major phospholipid, has been reported to increase brain levels of glucose and cAMP and to enhance turnover and release of dopamine and acetycholine when administered to rats peripherally. Thus, one approach to compensate for age-related dysfunctions might be to enhance dopaminergic and cholinergic function with PS. Preliminary data reported by Drago, et al.(Neurobiol.Aging, 1981) indicated that PS improved acquisition and retention of passive and active avoidance tasks in aged rats.

Previous work in our lab and others has shown that aged rats demonstrate: 1)severe retention impairments on a single trial step-through passive avoidance task and 2)severe impairments on a battery of psychomotor tasks designed to measure muscle strength and tone, equilibrium, and psychomotor coordination. Using these tasks we tested the effects of PS in aged Fischer 344 rats(22 mo. of age). PS (12.5-50 mg/kg) was administered ip., 30 min prior to the behavioral tasks.

All doses of PS produced a significant increase in retention latencies (measure of memory) over control scores in a dose-related manner. This improvement could not be explained on the basis of changes in locomotor activity or foot-shock sensitivity. PS did not have any significant effect in psychomotor performance at any of the doses tested.

239.14 FACILITATION OF RETRIEVAL BY PRE-TEST ADMINISTRATION OF OXOTREMORINE IN MICE. H. J. Altman Lafayette Clinic, Detroit, MI 48207

Stimulation of the cholinergic nervous system with the potent and highly selective muscarinic cholinergic agonist oxotremorine (OXO) has been shown to facilitate retention of a one-trial inhibitory avoidance response in mice, when this compound was administered immediately following training (Baratti et.al., 1979; Huygen et.al. 1980). It was, therefore, suggested that stimulation of post-synaptic muscarinic receptors immediately following training enhanced the process(s) of memory consolidation and/or storage.

The purpose of the present series of experiments was to determine what effects, if any, direct muscarinic cholinergic receptor stimulation would have on the retention of a previously acquired aversive habit if OXO were administered prior to the retention test.

The behavioral task used was a modification of the standard one-trial inhibitory avoidance paradigm (Quartermain and Altman, 1982), in which thirsty mice were trained to drink from a tube in an experimental chamber and 24 hrs. later, following an initial period of drinking, were shocked (via the tongue), each time they touched the drinking spout (0.75mA/3shock max). Retention was measured as the latency to complete 5 sec. of drinking 48 hrs. later (under extinction conditions).

The results indicate that peripheral administration of OXO 30 min. prior to retention testing results in a significantly elevated suppression of drinking compared to saline injected controls ($t=2.34; P<0.05$). The facilitation by OXO was both dose and time dependent and blocked by pretreatment with scopolamine·HBr.

The longer latencies exhibited by the animals treated with OXO was not likely to have been due to non-specific effects of the drug on behavior in general, as the latencies of an independent group of non-contingently (NCS) shocked mice, (Quartermain and Altman, 1982), were very short ($t-4.86; P<.001$). That is, had the administration of OXO resulted in illness, a reduction in thirst or reduced locomotor activity the NCS trained animals, would have had long latencies. Since the latency scores of the animals in this group were short, the results suggest that OXO is enhancing the memory of the original avoidance habit.

The results of the present series of experiments suggest, therefore, that OXO can also facilitate retrival processes.

239.15 THE ROLE OF AMYGDALAR CHOLINERGIC ACTIVITY IN TASTE-POTENTIATED ODOR AVERSION LEARNING. F. Bermudez-Rattoni, F. Chavez-Alamanza* K. Coburn* and J. Garcia*. Department of Psychology, Mental Retardation Research Center, UCLA, Los Angeles, California 90024.

Previous reports have shown that odor and taste stimuli may be affected by different temporal parameters during toxiphobic conditioning. Odor must be followed immediately by poison, while taste alone is effective even with delayed poison. However, when odor plus taste is followed by delayed illness, odor becomes aversive as if it has been potentiated by taste. Our interest was in the neural mechanism involved in the potentiation of odor by taste. It has been shown that "reversible lesions" of the amygdaloid complex (using procaine) disrupt potentiated odor aversions, but not taste aversions. Also, it has been shown that cholinergic activity is highly involved in retention of learned aversion tasks. Thus, it is highly likely that cholinergic activity is involved in the gating of the odor into the taste system. The purpose of this experiment was to learn the role of the cholinergic mechanisms of the amygdala in potentiated odor aversions.

Rats implanted with bilateral cannulae in the amygdala received physiostigmine (phys; 9µg/3µL), scopolamine (scop;30µg/3µL) or saline (sal; 3µL) infused over 3 min. Almond odor and .1% saccharine were the CSs and .15M LiCl (190 mg/kg) was the US. One group of rats received the drugs before acquisition and another group received the same drugs before the test. During acquisition the drugs were given 30 min before the presentation of odor and taste (CSs) and were followed by delayed illness (US). Then the animals were tested with odor or taste alone in separate test days with water days in between. The rest of the animals received the same drugs 30 min before the presentation of odor or taste during the test days in a counterbalanced order.

Results of the manipulations showed that when given prior to acquisition, phys significantly decreased the odor aversion, while scop produced a nonsignificant increase. Taste aversions were unaffected. Administered prior to testing, scop increased odor aversion but not taste aversion; phys increased taste aversion and tended to decrase odor aversion. The general trend of these results is toward an inverse relationship between cholinergic activation in taste potentiated odor aversion learning.

Research supported by the following grants: National Institute of Health NS11618, Program Project Grant HD05958 and CONACYT 24142 (Mexico) to Federico Bermudez-Rattoni.

239.16 NON-CONTINGENT ACTION OF NATURAL REINFORCERS ON MEMORY. Claude Messier and Norman M. White. Department of Psychology, McGill University, 1205 Dr. Penfield Ave., Montreal, Canada, H3A 1B1.

Previous studies have shown that retention of a learned behavior is improved by a post-training and non-contingent session of self-stimulation (Coulombe & White, Physiol. Behav., 25 :267-272, 1980; Can. J. Psychol., 36 :57-66, 1982). The facilitative effect of self-stimulation was interpreted as a non-contingent strenghtening action of that reinforcer on the previously formed association. Although the self-stimulation technique has proved useful in helping to understand the neural basis of reinforcement, it is a highly artificial event. Therefore, the present experiments sought to replicate the self-stimulation findings using laboratory versions of "natural reinforcers": sweet solutions of sucrose and saccharin.

Preference curves were generated by testing 14 concentrations each of sucrose and saccharin in a 20-minute test in which rats were presented with a choice of a sweet solution and water. Three concentrations of each substance (the most prefered and one higher and one lower concentration) were chosen for further study. In the memory test, training consisted of pairings of a tone and a shock. The animals were allowed to drink one of the solutions after training. The next day, the tendency of the tone to suppress drinking was tested.

Post-training ingestion of the sucrose solutions but not of the saccharin solutions retroactively and non-contingently improved retention of the previously formed, classically conditioned tone-shock association. This suggests that the sweet taste of the solutions was not the cause of the improvement in retention. The hypothesis that some post-ingestional effects of sucrose caused the effect was supported by the observation of improved retention with post-training subcutaneous (sub-q) injection of glucose (1 ml of a 30g/100 ml solution). An equivalent or doubled amount of fructose (a sugar that does not cross the blood brain barrier but is metabolized in the liver) had no effect when injected sub-q. This suggest that some central action of glucose may cause the improved memory.However, intraventricular injections of glucose (5 ul of a 30g/100 ml solution) also had no effect on retention.

This series of experiments parallels previous findings obtained with self-stimulation as the reinforcer. The results support the hypothesis that reinforcers produce a non-contingent, retroactive enhancement of retention of previously formed association. Further investigation will be necessary to discover the route by which glucose has this effect.

239.17 LEUPEPTIN, AN INHIBITOR OF CALCIUM ACTIVATED PROTEINASES, SELECTIVELY DISRUPTS MEMORY. U. Staubli*, M. Baudry and G. Lynch. (SPON: J. Conway). Department of Psychobiology, Univ. of California, Irvine, CA 92717.

Biochemical studies have indicated that the number of glutamate receptors in membranes isolated from mammalian forebrain is irreversibly increased by the activation of a calcium sensitive proteinase ("calpain"). It has been suggested that this process is involved in the formation of those types of memory that require hippocampus and neocortex.

To test this idea, we measured the effects of leupeptin, a potent inhibitor of the proteinase, on several memory tasks in rats. Leupeptin or saline was injected into the lateral ventricle with implanted osmotic minipumps at the rate of 10 µg/hr over a period of 14 days. The drug caused no apparent changes in food and water intake or in body temperature. However, leupeptin treated rats (n=8) committed many more errors than did controls (n=7) in a 8 arm radial maze task that involved a 15 minute delay between the 4th and 5th choices ($p < 0.001$).

Separate groups of rats were also tested on a Y maze problem. In this, the animals choose one of two arms for a food reward and are then removed to a holding cage for 15 min to 2 hours. They are returned to the start box with the reward placed in the arm not entered on the first trial. Leupeptin treated rats (n=5) performed in an essentially random fashion on the second trial while controls (n=5) almost invariably made the correct response. Leupeptin did not disrupt all memory-related behaviors. Drug treated animals could be trained to go to a single arm of an eight arm maze for food reward over a period of several trials; moreover, they retained information about the task for at least 24 hours. Animals injected with leupeptin (n=5) were also indistinguishable from controls (n=5) in learning to escape from a mild shock and were only slightly slower in acquiring an avoidance response.

These results satisfy the prediction that inhibition of calcium activated proteases will interfere with certain types of memory. However, leupeptin blocks proteolytic enzymes other than calpain and it is possible that this effect contributed to certain of the results reported here. Behavioral experiments involving additional manipulations of the calcium proteinase are now in progress. (Supported by NIMH grant 197939-12 and NIA grant AG-00538. U.S. is supported by the Swiss National Science Foundation.)

LEARNING AND MEMORY: PHARMACOLOGY OF CONDITIONING

240.1 NALOXONE ENHANCEMENT OF MEMORY PROCESSES: DEPENDENCE ON INTACT NOREPINEPHRINE FUNCTION. P.R. Rapp and M. Gallagher. Dept. of Psychology, Univ. of North Carolina, Chapel Hill, NC 27514.

Opiate receptor mechanisms in both the soma-dendritic and terminal regions of norepinephrine (NE) neurons are capable of inhibiting NE function. The present research investigated the possibility that such interactions contribute to regulating memory processes. Numerous studies, including our own, have demonstrated that opiate agonists such as morphine can impair retention of recent learning. It is also well documented that interference with opiate receptor activation by administration of opiate antagonists can improve retention when comparable testing procedures are used. The effect of opiate antagonists on memory processes may be due to release of NE neurons from inhibition by opioid peptides. This suggestion has received some support from the finding that opiate antagonist enhancement of memory in rats can be prevented by administration of the β-adrenergic antagonist propranolol (Izquierdo and Graudenz, Psychopharmacology 67: 265, 1980). The present investigation examined whether the effects of opiate antagonists on memory processes might be altered by denervation of brain NE systems.

Three weeks prior to behavioral testing Sprague-Dawley rats received 6-hydroxydopamine (6-OHDA) or vehicle alone injected into the dorsal noradrenergic bundle. During behavioral testing rats received one-trial step-through passive avoidance training. Intraperitorial administration of the opiate antagonist naloxone or saline vehicle occured immediately after training. Retention for the training experience was conducted 24 hrs later. Animals were sacrificed 1 week following behavioral testing and brain catecholamine levels were assessed via radioenzymatic assay.

In agreement with previous reports post-training naloxone administration was observed to facilitate retention of passive avoidance training. Depletion of forebrain NE via 6-OHDA administration, however, blocked the enhancing effects of the opiate antagonist. Further evidence that the effects obtained in this investigation reflected dependence on NE activity is suggested by the observation that partial protection of brain NE neurons via desimpramine injection prior to 6-OHDA administration restored the memory enhancing effects of naloxone administration.

The results of the present investigation provide further support for the proposal that opiate receptor mechanisms may, at least in part, alter memory processes by regulating brain NE function. Further experiments are being conducted to investigate whether significant NE/opiate interactions occur within the amygdala, a brain site which has been demonstrated to be sensitive to NE and opiate treatments on memory processes. Supported by NIMH Grant MH35554 and NIMH Research Scientist Development Award to M.G.

240.2 EFFECTS OF MORPHINE, ETHYLKETOCYCLAZOCINE AND N-ALLYNORMETAZOCINE ON ACQUISITION OF THE CLASSICALLY CONDITIONED NICTITATING MEMBRANE RESPONSE. C. W. Schindler*, M. R. Lamb*, I. Gormezano* and J. A. Harvey. Departments of Psychology and Pharmacology, The University of Iowa, Iowa City, IA 52242.

Based on studies in the chronic spinal dog, Gilbert and Martin (J. Pharmacol. Exp. Ther. 197:517, 1976) proposed a three receptor model for the action of morphine and morphine-like drugs. Morphine, ketocyclazocine and ethylketocyclazocine (EKC), and N-allynormetazocine (NANM) were proposed to interact with the mu, kapa and sigma receptors respectively. Previous work in our laboratory has shown that morphine will profoundly retard the acquisition of the classically conditioned rabbit nictitating membrane response (NMR). Therefore the purpose of the present study was to determine the effects of the proposed kappa and sigma receptor agonists EKC and NANM on the acquisition of classically conditioned responses.

Classical conditioning of the rabbit NMR involved the presentation of tone and light conditioned stimuli (CS) for 800 msec before delivery of the unconditioned stimulus (UCS), consisting of a 100 msec msec electric shock to the skin over the paraorbital region of the head. Both 1 mg/kg (i.v.) EKC and 5 mg/kg morphine injected just prior to each conditioning session significantly retarded the acquisition of conditioned responses across 10 conditioning sessions of 60 CS-UCS pairings each. Both tone and light CSs were affected equally. The retardation produced by EKC and morphine was evident in measures of percent conditioned responses, average response latency and percentage of rabbits reaching a criterion of 10 consecutive conditioned responses. The retarded acquisition of conditioned responses produced by EKC and morphine could still be detected when the rabbits were tested five days after cessation of drug injections, suggesting that EKC and morphine were affecting acquisition and not performance of conditioned responses.

We have previously shown that 1 mg/kg naloxone has no effect on the acquisition of conditioned responses and the present results indicated that NANM and naloxone + NANM have only a small retardation effect on acquisition. However, both naloxone and NANM will block the retardant effects of either EKC or morphine on acquisition. These results suggest that the retardant effects of both EKC and morphine are due to an action at a single receptor site. This site could be the mu receptor proposed by Gilbert and Martin since both naloxone and NANM are mu recpetor antagonists. This suggests that EKC is a partial mu agonist as well as a kappa agonist.

Supported by grants DA 01759, MH 16841 and F32 DA 05245.

240.3 SEPARATE ROLES FOR NUCLEUS ACCUMBENS AND CAUDATE IN ACQUISITION AND PERFORMANCE OF AVOIDANCE BEHAVIOR. A. G. Phillips, J. MacCrea* and H. C. Fibiger. Departments of Psychology and Psychiatry, University of British Columbia, Vancouver, Canada.

Lesions of the ascending forebrain dopamine pathways or blockade of dopamine receptors with neuroleptic drugs prevent the acquisition of one-way avoidance behavior (Fibiger, Phillips and Zis, 1974; Fibiger, Zis and Phillips, 1975). However, these treatments do not disrupt the performance of this response in overtrained rats. The question remains as to whether response acquisition is dependent upon the integrity of the nigrostriatal and/or mesolimbic dopamine pathways. Temporary blockade of each of these pathways was produced by local microinjection of haloperidol (0.5, 5.0 µg) bilaterally into either the caudate nucleus or nucleus accumbens. In half of the experimental subjects, the drug injections were made prior to training in a one-way avoidance shuttlebox. A 10 sec tone signalled a subsequent foot shock (1.0 mA) and trials continued to a criteria of 8/10 avoidance responses or a 40 trial cutoff. On the second test session, those animals trained after vehicle injections now received haloperidol and the animals previously treated with haloperidol received the vehicle. Acquisition was blocked by microinjection of haloperidol (5µg) into either the caudate or accumbens. In contrast, a similar caudate injection had no effect on the second test session after acquisition of avoidance behavior. Accumbens injections did disrupt behavior on the second session despite successful acquisition on the preceding day. Ventricular injections of haloperidol (5µg) disrupted acquisition but not performance thereby replicating the pattern seen with caudate injections. Together, these data suggest that the dopaminergic input to the caudate is necessary for the acquisition of avoidance behavior but not the subsequent performance of this response. Integrity of the dopaminergic innervation of the accumbens appears to be important for the initiation of motor activity related to both the acquisition and performance of a voluntary avoidance response.

References

Fibiger, H.C., Phillips, A.G. and Zis, A.P. Pharmacol. Biochem. Behav. 2, 87 (1974).

Fibiger, H.C., Zis, A.P. and Phillips, A.G. Eur. J. Pharmacol. 30, 309 (1975).

240.4 ENHANCEMENT OF MOTOR CONDITIONED INHIBITION BY MICROINJECTIONS OF APOMORPHINE IN THE CAUDATE NUCLEI OF CATS. C. Barajas-López*, C. Reyes-Vázquez and H. Brust-Carmona. Depto. de Fisiología. Fac. de Medicina, Div. de Investigación, UNAM, México, D.F., México.

Dopamine (DA) microinjected into the Caudate Nuclei (CN) enhances the acquisition of an inhibitory motor conditioned response (IMCR), whereas haloperidol diminishes it. Furthermore, depletion of catecholamines, produced by the application of high doses of 6-OHDA into both CN, impairs this learned response. It has been suggested that the IMCR depends upon the functional integrity of the CN's aminergic systems. Hence, Apomorphine (AP), which might be a dopaminergic agonist, should facilitate IMCR. Cats were trained to press a lever (MCR) to obtain 0.5 ml of milk while a small light was on during 60 s; no reinforcement was given when it was off and paired to a sound during 20 s (IMSR), i.e., the animals had to learn to suppress the MCR. This paradigm was repeated 12 times in one session per day. After 3-5 sessions, under pentobarbital anesthesia we implanted stereotaxically a cannula into each CN at coordinates A 16, L 4.5, and H +4. After 4 retraining sessions, each animal received 5 bilateral microinjections (MI) of 10 or 20 µg of AP, one per session, alternated with sham microinjections. Both manipulations were performed 10 min before the sessions in an adjoining laboratory. A third group of cats -control- was only conditioned and tested at the same days as the treated animals. At the end of the experiments, under deep anesthesia the brains were perfused, and later on, histological sections (100 µm width) were made to localize the cannulae's position. During the IMCR, the average lever pressing tends to diminish in the control cats after repeating the sessions. In those treated with 10 µg of AP, the average lever pressing diminishes clearly with the first three MIs. After the 3rd MI the average lever pressing stays below the control level during the following sessions, being the difference statistically significant at $P<0.05$ (Mann Whitney U Test). In the group treated with 20 µg of AP, the average lever pressing diminishes very clearly during the first 4 microinjections, reaching s significant difference at $P<0.05$ (Mann Whitney U Test), but returns to the initial level during the sham microinjections. During the MCR, the average lever pressing of the control group tends to increase while repeating the conditioning sessions; whereas it diminished in the treated animals after the AP microinjections. Similar effects were described for DA microinjections into CN, except for the diminution during MCR. These findings further support the postulation that catecholamines in the CN have a behavioral inhibitory action upon a motor conditioned response.

240.5 EFFECTS OF CHOLINE INJECTIONS INTO THE CAUDATE NUCLEUS ON ACTIVE AVOIDANCE. R. A. Prado-Alcala, G. Cepeda*, L. Verduzco*, A. Jimenez* and E. Vargas*. Sch. of Medicine, Nat. Univ. of Mexico, Mexico 20, D.F. and Sch. of Psych., Anahuac Univ., Mexico.

Injections of acetylcholine-receptor blockers into the caudate nucleus (CN) significantly impair the acquisition and maintenance of instrumental behaviors. In line with these findings are those showing that small doses of choline injected into the CN improve the performance of lever pressing and passive avoidance behaviors. Some authors have reported, however, that cholinergic activation of the caudate disrupts avoidance performance. The present experiment was designed to test the idea that injections of a small dose of choline into the CN can facilitate active avoidance performance. During training male Wistar rats were put inside a shuttle box, and after 10 sec a tone and a light were turned on; if the rat jumped to the opposite compartment within 20 sec an avoidance response was computed; if the rat did not jump in 20 sec a 2.0 mA scrambled footshock was presented and remained on until the rat escaped to the opposite compartment. There were 10 training sessions of 40 trials each. To avoid a "ceiling effect" only rats with less than 50% avoidance responses during the last three training sessions were studied. After training cannulae were bilaterally implanted in the dorsal-anterior CN or in the dorsal-posterior CN. An additional 10 sessions were programmed. Bilateral injections of choline (15 ug/3 uL) were given to the implanted rats, and 100 mg/kg of choline (I.P.) to an unoperated group, 6 and 40 min before sessions 13 and 19, respectively. Saline solution (NaCl) was injected intracranially or I.P. 6 or 40 min before the 16th session. It was found that choline injections into the posterior CN significantly improved active avoidance performance relative to all other groups and treatments; the injection of choline into the anterior CN induced a better performance than intracranial injections of NaCl and I.P. injections of choline or NaCl. These results further support the hypothesis that cholinergic activity of the caudate nucleus mediates the performance of instrumental behaviors.

240.6 CENTRAL,BUT NOT PERIPHERAL,CHOLINERGIC INHIBITION PREVENTS HIPPOCAMPAL AND SEPTAL CONDITIONED UNIT RESPONSES AND RETARDS NICTITATING MEMBRANE CONDITIONING. S.D. Berry and A.T. Salvatierra*. Dept. of Psychology, Miami University, Oxford, OH 45056.

To clarify further the role of the septohippocampal system in learning, we recorded both EEG and multiple unit activity from the lateral septum and hippocampus after administration of an anticholinergic drug known to impair nictitating membrane (NM)conditioning.

Each of 16 New Zealand White rabbits was implanted with chronic recording electrodes in area CA1 of the dorsal hippocampus and in the dorsal lateral septal nucleus. Animals were given daily training sessions consisting of 13 blocks of 9 trials (8 paired;1 CS-alone). The CS was a 350 msec, 85 dB, 1KHz tone and the UCS was a 100 msec, 210 g/cm^2 corneal air puff occurring 250 msec after CS onset. Animals were trained to a criterion of 8 CR's in 9 trials or for a maximum of 4 sessions. Eight animals received scopolamine hydrobromide (HBR) and 8 received scopolamine methylbromide (MBR) as a peripherally-acting control. Drugs were given subcutaneously (1.5 mg/kg) 12 min before each session. Behavioral and Neural responses were recorded on magnetic tape for off-line analyses.

Baseline and postdrug EEG samples were analyzed with a zero crossing program and indicated that HBR produced significant shifts in EEG frequencies in the 1-12 Hz range in both structures, while MBR did not. Behaviorally, the HBR group took significantly longer (mean= 329 trials) than MBR to reach criterion (mean=120).

Analysis of unit activity consisted of passing filtered (500-5K Hz) records through a window discriminator and integrating the output in 10 msec segments, then averaging responses over 8 trials. While the MBR group developed the type of conditioned response characteristic of undrugged rabbits (Berger & Thompson, Brain Res., 1978, 145:323 and 156:293), the HBR group showed an almost complete suppression of learned unit responses in both hippocampus and lateral septum.

These findings corroborate relationships between hippocampal activity and learning rate in the rabbit NM paradigm and suggest that anticholinergic effects on learning may be mediated, in part, by a disruption of normal septohippocampal responses.

240.7 INCREASED GLUTAMATE RECEPTOR BINDING IN HIPPOCAMPUS FOLLOWING CLASSICAL CONDITIONING OF THE RABBIT EYELID RESPONSE.
R.F. Thompson, L.A. Mamounas, G. Lynch and M. Baudry. Dept. of Psychology, Stanford Univ., Stanford, CA 94305, and Dept. of Psychobiology, Univ. of Calif. Irvine, Irvine CA 92717.

During classical conditioning of the rabbit eyelid response, hippocampal pyramidal cells increase their rate of within-trial discharge. Long-term potentiation (LTP) is a candidate mechanism underlying this rapid, incremental and learning-dependent increase in neural activity. Similar cellular mechanisms may therefore mediate both LTP and the hippocampal neuronal changes associated with eyelid conditioning. The induction of LTP in hippocampal slices results in an increase in the number of glutamate binding sites in the potentiated region. The present study examines the kinetics of glutamate binding to hippocampal synaptic membranes following eyelid conditioning in the rabbit.

^{3}H-glutamate binding to hippocampal synaptic membranes was measured in three groups of rabbits: 1) a conditioning group that received paired presentations of the tone CS and corneal airpuff UCS; 2) a control group that received explicitly unpaired tone and airpuff presentations; and 3) a naive control group that received no handling and remained in the home cage until sacrifice. Paired animals were trained for three days -- a two hour training session per day. On the fourth day, they were given a thirty minute training session and were then returned to the home cage. One hour later, animals were decapitated and hippocampal synaptic membranes were prepared. Unpaired animals received identical amounts and sequences of unpaired sessions.

Animals receiving paired conditioning had significantly more glutamate binding sites (Bmax, 18.0 ± 1.8 pmol/mg protein) than either unpaired controls (12.6 ± 1.0) or naive controls (13.1 ± 1.3). There was a trend towards a decreased affinity (higher Kd) for the paired animals but this was not statistically reliable. The Hill coefficients for the three groups did not differ from unity. Several characteristics of ^{3}H-glutamate binding to rabbit hippocampal membranes and the regional distribution of binding sites within fields and layers of rabbit hippocampus indicate that, as in the rat, ^{3}H-glutamate binding is associated with a post-synaptic glutamate receptor.

Thus both repetitive electrical activation of hippocampal pathways which induces LTP and classical conditioning appear to produce increases in the number of glutamate receptors in hippocampus. This strengthens the argument that the neural changes in the hippocampus during eyelid conditioning are mediated by an LTP-like mechanism. (Supported by NSF # BNS-81-17115, ONR # NOOO14-83-K-0238 to RFT; NIMH # 1 T32 MH 17047-01 to LAM; NIMH # 19793-12 to GL; and NSF # BNS-81-12156-01 to MB.)

240.8 MICROINFUSION OF GABA ANTAGONISTS INTO THE CEREBELLAR DEEP NUCLEI SELECTIVELY ABOLISHES THE CLASSICALLY CONDITIONED EYELID RESPONSE IN THE RABBIT. L.A. Mamounas, J. Madden IV, J.D. Barchas and R.F. Thompson. Departments of Psychology and Psychiatry, Stanford University, Stanford CA 94305.

Discrete lesions of the dentate-interpositus region of the cerebellum have been shown to abolish selectively the well-conditioned, ipsilateral eyelid response in the rabbit (McCormick, et al, 1982). We are presently investigating the involvement of candidate cerebellar neurotransmitter systems in mediating this classically conditioned response. Several lines of evidence indicate that GABAergic processes are localized to these nuclear regions. Collectively, these observations provide an initial basis for examining the potential role of GABAergic processes in simple associative learning. We now report that microinfusion of GABA antagonists into the dentate-interpositus region of the cerebellum can produce complete, selective and reversible abolition of the well-conditioned eyelid response--with the unconditioned reflex response being unaffected by these manipulations.

These pharmacological effects behave in a dose-dependent fashion with 1.5 nmol (in 0.75 µL of vehicle) of either bicuculline methiodide or picrotoxin completely abolishing the ipsilateral conditioned eyelid response (CR) following drug administration. Lower doses produce a dose-dependent decrease in the CR peak amplitude. Typically this abolition dissipates over time--with CRs returning to predrug baseline levels by the end of the test session. This selective abolition by GABA antagonists can be produced regardless of the amount of prior training the animal has received. This effect appears highly selective based on its pharmacological specificity and anatomical localization of site of action. Several candidate agonists and antagonists thus far examined, including glycine and strychnine, produce no measurable effect on the conditioned behavior when administered in comparable molar doses. Histological verification of sites of infusion indicate that cannula tip placements are most effective in disrupting the CR if positioned immediate to the dentate-interpositus region. When training is shifted to the eye contralateral to the infusion cannula, bicuculline infusions (1.5 nmol/ 0.75 µL) are completely ineffective in disrupting the contralateral CR. This argues against the substance diffusing via the general circulation and producing its effect at distant sites.

Collectively these results suggest that GABAergic processes localized to the deep cerebellar nuclei may play a critical role in simple associative learning.
Supported by NIMH # 1 T32 MH 17047-01 to LAM; NIMH # MH 23861 to JM IV and JDB; NSF # BNS-81-17115, ONR # NOOO14-83-K-0238 to RFT.

240.9 MICROINFUSION OF PICROTOXIN INTO THE CAUDAL RED NUCLEUS SELECTIVELY ABOLISHES THE CLASSICALLY CONDITIONED NICTITATING MEMBRANE/EYELID RESPONSE IN THE RABBIT. J. Madden IV, D.A. Haley, J.D. Barchas and R.F. Thompson. Depts. of Psychology and Psychiatry, Stanford University, Stanford CA 94305.

A wealth of experimental evidence suggests that cellular processes within the dentate-interpositus region of the cerebellum play a critical role in the expression of at least some forms of simple associative learning, including the short-delay classically conditioned nictitating mebrane (NM)/eyelid reponse in the rabbit (McCormick, et. al., 1982, Mamounas, et. al., 1983). More recently, Haley, et. al. (1983) have observed that lesions of the contralateral red nucleus, a structure receiving major efferent projections from the dentate/interpositus region of the cerebellum, also abolish the conditioned contralateral NM/ eyelid response. These observations provide a basis for postulating that this stucture may also be an essential component of the conditioned response pathway. In an attempt to further examine this possibility, we have assessed the effects of pharmacological manipulations of this region on the expression of the conditioned response. We now report that microinfusion of 1.75 nmol of picrotoxin (in 0.75 uL of vehicle) into the caudal red nucleus selectively abolishes the conditioned NM/eyelid response--the unconditioned reflex response is essentially unaffected by this manipulation. Furthermore, this effect can be produced regardless of the amount of prior training the amimal has received. Collectively these results and those of Haley, et al (1983) suggest that the red nucleus is an essential component of the conditioned response pathway in this simple form of associative learning.
Supported by NIMH # MH 23861 to JM IV and JDB; NSF # BNS-81-17115, ONR # NOOO14-83-K-0238 to RFT.

240.10 BEHAVIORAL MEASURES AFTER CENTRAL NORADRENERGIC DEPLETION WITH THE NEUROTOXIN, DSP-4: LACK OF IMPAIRMENT IN AVOIDANCE TASKS.
C. Bennett*, J.L. McGaugh, M. Arnold*, and K.C. Liang*. (Spon: G. Novack) Department of Psychobiology, University of California, Irvine, CA 92717

The behavioral effects of depletion of central norepinephrine (NE) by a neurotoxin specific for noradrenergic terminals, N-2-chloroethyl-N-ethyl-2-bromobenzalamine (DSP-4), were investigated by a variety of measures. In previous behavioral studies, lesions of the central NE nuclei or pathways have produced mixed results. In the present study we examined inhibitory avoidance retention, active avoidance acquisition and retention, activity, and shock sensitivity after central NE depletion with DSP-4.

Male Sprague-Dawley rats were injected with 50 mg/kg (ip) of DSP-4 synthesized by two of us (Arnold and Bennett), according to the method of Krueger and Cook (1975). This dose has been shown to deplete central and peripheral norepinephrine (NE) by 60-90%, depending upon the region. Behavioral measures were begun 14 days after the injection to allow regeneration of the peripheral sympathetic terminals. The rats were trained on a one trial step-through inhibitory avoidance task using a training footshock of either 0.75 mA, 1 s, or 1.0 mA, 2 s. Retention was tested 24 hours later. After two weeks, the rats were trained on an active avoidance task (0.64 mA, 30 s) to a criterion of three avoidances. Retention was measured by retraining the animals to the same criterion after 24 hours. Animals not reaching the criterion within 15 trials on the training day were not tested for retention.

For the inhibitory avoidance task, the two groups did not differ in their latencies to step through on the retention test. The DSP-4 treated rats, however, had significantly longer mean entrance latency on day-1 than the controls. In the active avoidance task, there was no significant difference between the two groups in the number of trials to criterion for acquiring the response, nor was there a significant difference in the retention scores. We also measured spontaneous activity by counting line crossings and rearings in an open field apparatus. There were no significant differences between the two groups, although the control group had higher mean values for both measures. Flinch-[illegible] lysis revealed that the two groups also had comparable thresholds for both flinch and jump, indicating that there is no difference in shock sensitivity.

In a variety of measures, therefore, DSP-4 treated animals appear remarkably similar to control animals. While acute pharmacological treatments affecting NE are known to influence behavioral measures of learning and memory, our findings suggest that functionally intact central NE systems are not essential for normal acquisition and retention of avoidance tasks.
Supported by USPHS grants MH 12526 and AG 00538 (to JLMcG).

240.11 YOHIMBINE'S ATTENUATION AND CLONIDINE'S FACILITATION OF SHORT-TERM HABITUATION OF THE ACOUSTIC STARTLE RESPONSE IN RATS. James V. Cassella and Robert N. Leaton. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Previous research has shown that although many drugs modulate the amplitude of the acoustic startle response (ASR) in rats, only clonidine unambiguously alters short-term habituation of the response (Davis et al.,1977). Given clonidine's pharmacological specificity for NE alpha-2 receptors it was hypothesized that this class of NE receptors plays an important role in this habituation process. Accordingly, it was expected that the NE alpha-2 antagonist, yohimbine, would block clonidine's action on the ASR. Moreover, yohimbine itself was expected to attenuate short-term startle habituation. The present series of experiments explored the effect of yohimbine, as well as clonidine, on short-term habituation of the ASR.

In Experiment 1, yohimbine treatment (10, 15, 18 mg/kg, i.p.) significantly attenuated the short-term response decrement to 20 startle- eliciting tones (4-kHz, 118-dB, 100-ms duration, 20-s ISI). Contrary to previous observations, however, clonidine (.03 mg/kg) failed to accelerate the rate of habituation. The absence of a clonidine habituation effect might be attributed to a floor effect resulting from the significant depression of first-trial startle amplitudes in these animals.

In Experiment 2, first-trial startle amplitudes following clonidine treatment (.03 mg/kg) were comparable to control levels and, more importantly, habituation was accelerated. As predicted, pretreatment with yohimbine (1, 3, 10 mg/kg) significantly blocked clonidine's habituation effect.

Experiment 3 found that clonidine's facilitation and yohimbine's attenuation of ASR habituation were dependent upon the parameters of stimulation (white noise bursts), especially the combination of stimulus intensity and ISI. Clonidine's habituation effect was most consistently observed under conditions in which habituation in control animals was relatively weak, namely at a high stimulation intensity (110-dB) and/or long ISIs (60-s). Conversely, yohimbine's habituation effect was most consistently found when habituation in controls was relatively strong : at a low or moderate intensity of stimulation (90-100 dB) and/or short-to-moderate ISIs (10-30-s).

Given the antagonistic nature of clonidine and yohimbine on startle habituation and the opposing pharmacological actions of these drugs, the data suggest that NE alpha-2 receptors might play an important role in the mediation of short-term habituation of the ASR.

240.13 ENHANCING EFFECT OF EPINEPHRINE ON RETENTION OF AN INHIBITORY AVOIDANCE RESPONSE: LACK OF ATTENUATION BY DEXAMETHASONE. K. C. Liang*, C. Bennett*, J. L. McGaugh, D. A. Tam, Jr.*, and R. G. Juler*. Department of Psychobiology, University of California, Irvine, CA 92717.

Considerable evidence has shown that posttraining systemic injections of epinephrine or adrenocorticotropin (ACTH) enhance retention in various learning tasks. Recent studies indicate that epinephrine, given systemically, may act on the anterior pituitary and release ACTH. These findings suggest that epinephrine may enhance memory by way of releasing ACTH. According to this hypothesis, dexamethasone, which blocks the epinephrine-elicited ACTH release, should attenuate the enhancing effect of epinephrine on memory. The present study addressed this question.

Forty-nine male Sprague-Dawley rats received two subcutaneous injections (16 hrs apart) of dexamethasone (Dex, 2 mg/kg) or vehicle (Veh). Four hrs after the second injection, animals were trained on a one-trial step-through inhibitory avoidance task with a 600 µA, 0.5 sec footshock. Immediately after training, rats received an intraperitoneal injection of epinephrine (Epi, 0.1 mg/kg) or saline (Sal). Retention was tested 24 hrs later.

Retention performance is shown in the table. In the Veh pre-treated rats, posttraining Epi significantly enhanced retention ($U=39.5$, $p < 0.02$). The two groups of Dex pre-treated rats, when combined, had significantly longer retention latencies than the combined Veh pre-treated group ($U=172$, $p < 0.02$). The most critical finding is that in the animals pre-treated with Dex, posttraining Epi still caused a significant enhancing effect on retention ($U=24.5$, $p < 0.05$).

These results indicate that dexamethasone, which blocks the ACTH release elicited by epinephrine, failed to attenuate the retention-enhancing effect of epinephrine. These findings do not support the hypothesis that epinephrine enhances memory by way of releasing ACTH.

Pretraining treatment	Veh		Dex	
Posttraining treatment	Sal	Epi	Sal	Epi
Median retention latencies	193.9 (13)	548.1** (14)	370.3 (10)	600* (12)

** $p < 0.02$, * $p < 0.05$ different from the saline groups, two-tailed Mann-Whitney U-tests. ( ) = number of animals.

The present study is supported by US Public Health Service Research Grants MH 12526 and AG 00538 (to JLMcG).

240.14 **TIME OF DAY IS IMPORTANT IN THE RETRIEVAL OF ENHANCED MEMORY FOR PASSIVE AVOIDANCE BEHAVIOR BY POST-TRAINING ETHANOL IN MICE.** **D.L. Colbern, and E.G. Zimmermann. Brain Research Institute and Department of Anatomy, UCLA School of Medicine, Los Angeles, CA 90024.**

Passive avoidance measured 24 h, 5 or 7 days later, is improved when mice are injected with ethanol immediately after footshock training in the afternoon (Alkana and Parker, Psychopharm., 66:117-119, 1979; Colbern, et al, Substance and Alcohol Actions/Misuse, 1:181-186, 1980). However, avoidance performance is not improved when mice are trained and injected with ethanol in the morning and tested 24 h later (Colbern, et al, Alcoholism: Clin. Exp. Res., 5:146, 1981).

In mice, the diurnal variation of ethanol-facilitated avoidance closely resembles the circadian pattern of corticosterone release during light hours. Endogenous levels of corticosterone are low in the morning and rise to a peak in the afternoon (Kakihana and Moore, Psychopharmacologia, 46:301-305, 1976), while the 3 g/kg dose of ethanol used in our studies produces maximal release of corticosterone (Kakihana, et al, Nature, 218:360-361, 1968). Glucocorticoid states after training and before testing may be an important condition for the retrieval of information regarding the training experience.

In a study designed to behaviorally determine if high afternoon levels of corticosterone might be necessary for the observation of enhanced avoidance, male Swiss-Webster mice were given passive avoidance training (0.1 mA) in the late afternoon and then immediately injected with ethanol (3.0 g/kg, 15 % v/v). Controls were given an equal volume of saline or a sham injection. Avoidance testing was conducted 24, 38, or 48 h later.

For all treatment groups, mice tested in the afternoon, 24 or 48 h after training, exhibited more avoidance behavior than did mice tested in the morning, 38 h later. However, mice injected with ethanol showed greater avoidance at these times than did mice given saline or sham injections. Thus, the similarity of endogenous states at times of training and testing was important in the retrieval of information for all mice. It appears that the effect of post-training ethanol on passive avoidance behavior is to potentiate normally occurring processes.

Supported in part by USPHS Grant NIAAA-03513 to E.G.Z.

240.15 SUPPRESSION OF MEMORY EXPRESSION BY PUROMYCIN AND ANTAGONISM BY CLYCLOHEXIMIDE. R.A. Barraco, H. Normile*, G. Smoot*, H. Altman. Wayne State University, School of Medicine, Detroit, MI 48201.

Two types of interpretation have been enunciated to account for deficits during retention: either the memory no longer exists or the memory cannot be adequately retrieved. Impairment of retrieval processes by an amnestic agent can be substantiated when a given memory is recovered by amnesia-attenuation treatments. Although the memory-impairing effects of the majority of antibiotics are generally correlated with the temporal parameters of protein synthesis inhibition and the training-treatment interval, the situation for puromycin (PM) is very different. PM injections impair retention when they are made before or immediately after training or when they are delayed until 24 or more hrs after training. Further, although there is no evidence of memory restoration or amnesia attenuation for PM injections given immediately after training, the amnesia produced by delayed PM injections can be attenuated by a variety of manipulations (Barraco, R.A. and L.J. Stettner, Psychol. Bull. 83: 242, 1976).

Mice were trained in a lick-suppression task and given intracerebroventricular (ICVT) injections of PM 24 hrs training. PM produced a marked amnesia for the inhibitary avoidance response during retention testing 96 hrs later. Of all the antibiotics used in memory disruption experiments, only PM is effective as an amnestic agent when injections are delayed until 24 hrs after training. In another experiment, mice were trained in the same task and given concurrent ICVT injections of PM and cycloheximide (CXM). At the higher dose, CXM completely blocked the amnestic effects of PM. These results suggest that delayed PM injections block the retrieval or expression of memory. The fact that CXM does not attenuate the amnesia produced by immediate PM injections suggests the biochemical mechanisms for amnesia in each situation is not the same. Furthermore, since CXM blocks the formation of a fraction of puromycin peptides which has been isolated and shown to persist in the mouse brain for at least 96 hrs, the amnesia-attenuating effects of CXM strongly implicate the involvement of this persistent fraction of puromycin metabolites in blocking the expression of memory. We are now examining the bioactivity of these persistent puromycin metabolites using a number of in vitro and behavioral methods.

241.1 INDUCED REDUCTION IN FIBER NUMBER OF INDIRECT FLIGHT MUSCLES IN THE DROSOPHILA MUTANT SHIBIRE. W.J. Costello and L. Salkoff, Dept. Biomed. Sci./Col. Osteo. Med., Ohio Univ., Athens, OH 45701 and Biol. Dept., Yale Univ., New Haven, CT 06511.

The genetic approach to explain problems in development can be conducted with great success in studies utilizing the fruit fly Drosophila melanogaster. One mutant, shibire^{ts1}(shi) is temperature-sensitive, causing reversible paralysis of flies at temperatures above 28°C. As well, a variety of phenotypic responses to heat pulses are expressed at different developmental stages (Poodry et al(73)Dev. Biol. 32:373-386). Several morphological defects are seen in these heat-pulsed flies. Synapses contain few or no vesicles but do have numerous membrane-bound cisternae (Poodry & Edgar(79)J. Cell. Biol. 81:520-527). There are also cisterna-like structures seen in close association with the sarcolemma of the indirect flight muscles (Costello & Salkoff(82) Neurosci. Abstr. 8:494). Evidence suggests that shi may be causing an overall membrane defect affecting turnover and re-cycling.

We have found that the shi defect also prevents normal fiber formation of the dorsal longitudinal muscles (DLM) during pupal development. During normal development, adult myocytes in the early pupa ($\leq$10hrs) migrate to a set of larval muscles in the dorso-lateral region on either side. The myocytes organize themselves around the larval muscles and commence to fuse. Concurrently, the larval muscles undergo complete degeneration. The fused myocytes form into three units corresponding to the three larval muscles. Each unit then divides in half so that by 24 hrs of pupation there are six units formed. These six units are the precursors to the six DLMs of the adult.

In our experiments, shi flies were raised at 18°C. Pupae of various ages were heat-pulsed at 30°C for 2 hrs. Afterwards, the pupae were returned to 18°C for the duration of pupal development. The newly emerged adults were dissected at 18°C and prepared with cold fixative. We found that in adult flies which had been heat-pulsed between 0-17hrs of pupation the DLM fiber number was abnormal. The DLM, though present, consisted of only three units on either side. Serial sectioning confirmed that the units maintained autonomy throughout the thorax. Ultrastructurally, the three DLM units appeared normal: myofilaments were organized in normal fashion and sarcomeres were present.

The evidence suggests that the shi defect is preventing normal boundary formation in the development of DLM fibers.

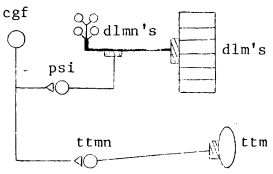
Supported by the Muscular Dystrophy Association and a grant from the Ohio Univ. Research Committee.

241.2 CHOLINE ACETYLTRANSFERASE MUTATIONS BLOCK ACTIVITY AT A CHEMICAL SYNAPSE IN DROSOPHILA MELANOGASTER. M. Gorczyca and J. Hall Dept. of Biology, Brandeis University, Waltham, MA 02254

Two temperature sensitive mutations of the choline acetyltransferase gene (Cha) have been used in the initial stage of identification of the neurotransmitter at a chemical synapse in the giant fiber motor circuit in Drosophila melanogaster.

These mutations, Cha^{ts1} and Cha^{ts2}, affect levels of choline acetyltransferase (CAT and acetylcholine (ACh). CAT activity declines to less than 2% after incubation at nonpermissive temperature for 3-4 days. Concomitantly, ACh levels are reduced to 25% wild-type (wt). This has been shown to severely affect courtship and locomotor activity as well as a transient spike in the electroretinogram.[1] Presumably, cholinergic neurons in the CNS are disturbed by the decrease in transmitter levels.

To circumvent the difficulties of recording directly from the CNS to assay neurophysiological abnormalities, a previously identified circuit in the fly was selected.[2] Access to both presynaptic and postsynaptic elements is possible. The giant fiber motor circuit mediates the animal's escape response which is channeled down two pathways: (1) cervical giant fiber (cgf) to peripherally synapsing interneuron (psi) to dorsal longitudinal muscle motorneurons (dlmn's) to the dorsal longitudinal muscles (dlm's) and (2) cgf to tergotrochanteral muscle motorneuron (ttmn) to the tergotrochanteral muscle (ttm).



Upon brain stimulation in wild-type flies, responses are evoked in the dlm's and the ttm. In both Cha mutants at restrictive temperature, the first pathway completely fails (no response) while the second pathway performs normally. The pathway from the dlmn's to the dlm's however, behaves as in wild-type flies. Hence, the psi is the aberrant component of the pathway. Since the synapse from the psi to the dlmn's is almost certainly chemical,[2] this suggests that acetylcholine is the neurotransmitter at that synapse.

A direct and positive correlation between diminution of CAT activity and the respose in the cgf-to-dlm pathway can be made using these mutations. It was found that CAT activity might be the limiting factor in the ability of these neurons to respond to high rates of stimulation.

1. Greenspan, R. J. Comp. Physiol. 137:83-92 (1980)
2. Wyman, R., Tanouye, M. J. Neurophys. 44:405-421 (1980)

241.3 BITHORAX COMPLEX MUTANTS TRANSFORM THE SEGMENT-SPECIFIC PATTERN OF 5-HT CONTAINING NEURONS IN DROSOPHILA. E. Martel*(SPON: K. Graubard). Dept. of Zoology, University of Washington, Seattle, WA 98195.

Certain aspects of a neuron's identity are a consequence of its segment of origin. In an effort to understand the genetic basis of this determination I have developed a segment-specific neuronal marker and used it to observe transformations of segmental identity in the central nervous systems of mutants of the Bithorax Complex. The fused segmental ganglia of adult Drosophila were stained using an antiserum produced against 5-Hydroxytryptamine (5-HT). Immunoreactive cell bodies were arranged in a segment-specific manner, with both the prothoracic and mesothoracic ganglia containing two pairs of cells and the metathoracic ganglia being devoid of staining. Immunoreactive cells are also seen in both larval and adult abdominal ganglia. The pattern of 5-HT staining in wild type flies is highly stereotyped.

Studies of the genetic basis of the determination of segmental identity in the epidermis of Drosophila have resulted in an understanding of the region of the genome known as the Bithorax Complex, which controls the identity of thoracic and abdominal segments. Loss of gene activity in this region is known to produce homeotic transformations, transforming the identity of epidermal cells to that of segments more anteriorly located. Application of antiserum to 5-HT on to the ventral nervous systems of Bithorax Complex mutants revealed apparently homeotic transformations of the pattern of staining similar to those transformations seen in the epidermis. In flies homozygous for the triple mutation abx bx^3 pbx, the metathoracic ganglia contained four immunoreactive cells arranged in the pattern seen in the mesothorax. Staining of double and single mutants reveals partial transformations. The pattern of 5-HT staining in all other segments is unchanged. This work indicates that the determination of segmental identity in the central nervous system is under the genetic control of the Bithorax Complex.

241.4 WIDESPREAD NEURONAL AND AXONAL DEGENERATION IN THE TORTURED, TOR, MUTANT MOUSE. C. F. Da Silva, J. C. COWEN* and R. L. Sidman. Depts. of Neuroscience, Children's Hospital and Neuropathology, Harvard Medical School, Boston, MA 02115.

An initial brief description (Sidman, in Kety et al. Genetics of Neurol. & Psychiat. Disorders, A.R.N.M.D., 60, 1983) of this new autosomal recessive mutation emphasized the grossly abnormal postures and adult-life progressive degeneration of cerebellar granule cell neurons. We now report widely distributed neuronal degeneration peaking at different times for different populations over a span of at least one year. At postnatal day 17 (P17), many dorsal root ganglion (DRG) neurons appear chromatolytic and others are abnormally small, though their peripheral and central axonal branches appear intact. At P27-50, Wallerian degeneration is widespread in motor and sensory peripheral nerves and roots, in all white matter funiculi at all levels of spinal cord, and in brainstem, but cerebellar white matter is only mildly affected and forebrain tracts appear normal. We recognized no distal-proximal gradients of degeneration along a given fiber system. Some medium- and large-sized neurons apparently had degenerated without residue in DRGs, ventral horns of spinal cord and in the cerebellar Purkinje layer and deep nuclei; these areas showed little further cell loss after the second month. By contrast, pyknotic and fragmented remnants of small cells were present in large numbers, peaking at about P30-50 in the caudate/putamen, P50 in nuc. accumbens septi and the polymorphic layer of the pyriform cortex, and later than P50 for periglomerular and granule cells of olfactory bulb and for cerebellar cortical granule cells. After the second month, Purkinje cells appeared to stabilize in number and increase in concentration as granule cells progressively disappeared and the cerebellum atrophied. Degenerating myelinated axons were first noted in both limbs of the anterior commissure and in the lateral olfactory tract at P50, and were more numerous at P79. A few degenerating fibers were observed in the optic nerve and chiasm at P223 and more were present at P373. The common feature among these neuronal populations that might suggest the mode of action of the tor genetic locus remains elusive: some affected neurons are large and others small, some central and others peripheral, some excitatory and others inhibitory; they share neither time of genesis nor time of degeneration.

Supported by Fellowship Award (200.202-81-BF) from the Conselho Nacional de Desenvolvimento Científico & Tecnológico (CNPq), Brazil to C.F.S., NIH Grant NS 11237 to R.L.S. and HD 06276.

241.5 THE ARCHITECTONIC AND HODOLOGIC ORGANIZATION OF THE REELER CEREBELLUM. A.Goffinet*, K.-F.So, V.S.Caviness Jr. Dept. Neurol.and Neuropathol., M.G.H. and E.K.Shriver Center, Boston, MA 02114.

The architectonic and hodological organization of the reeler cerebellum has been studied using cell and fiber stains, retrograde transport of HRP, anterograde autoradiographic labeling, and the Fink Heimer method. The reeler cerebellum is composed of six concentric zones: 1) a thin molecular layer,2) a sparsely populated Purkinje cell layer, 3) an irregular granular layer, also containing displaced Purkinje cells,4) a fiber layer, 5)a zone of heterotopic Purkinje cells, in which three masses (central, intermediate and lateral) are defined and 6)the roof nuclei. The Purkinje cell populations of the cortex and subcortex are continuous along two rostrocaudally aligned parasagittal planes. They serve to partition the hemicerebellum of the mutant into a midline and two more lateral rostrocaudally aligned compartments. Principal rhombencephalic afferent sources defined by intracerebellar HRP injection are, as in the normal animal, the inferior olivary nuclei, lateral reticular nuclei , trigeminal nuclei, cuneate nuclei, vestibular nuclei, locus coeruleus, raphe nuclei, pontine and reticulotegmentopontine nuclei. The inferior olive is less convoluted in reeler than in the normal animal (Goffinet,'83).Still the rostral to caudal axis of the cerebellar projection of the olivary complex is distributed continuously over the lateral to medial axis of the cerebellum as in the normal animal.The projection in reeler differs from that in the normal in that there is no apparent subdivision into sagittally aligned stripes in the mutant. Tracts carrying the mossy fiber projections are diminutive in reeler. Pontine afferents are distributed to the two lateral cerebellar compartments and terminate exclusively in the granule cell layer. Spinocerebellar afferents are limited to the medial compartment and terminate in the granule cell layer as well as among heterotopic Purkinje cell masses (Wilson et al.,'81). The pontine and spinal mossy fibers projections do overlap somewhat in the paramedian zone of Purkinje cells but principally they occupy differentially the paramedian and median cerebellar compartments, respectively. This study shows that the afferent connections of the reeler cerebellum are normally organized topologically but do include at least some classes of heterologous synapses. In addition, there is a correspondance between architectonic and hodologic compartments in the mutant, which is not found in the normal cerebellum.

241.6 SINGLE GENE INHERITANCE OF AN ABNORMALITY IN LAMINATION IN AREA CA3c OF THE HIPPOCAMPUS OF BALB/c MICE. R. S. Nowakowski. Dept. of Anatomy, Univ. Miss. Med. Ctr., Jackson, MS 39216.

In BALB/c mice the lamination of the pyramidal cell layer of area CA3c of the hippocampus is abnormal in that early-generated neurons are superficial and late-generated neurons are deep (Vaughn et al., '77, J. Comp. Neurol. 173: 41-52). This reversal from the normal pattern suggests that neuronal migration in BALB/c is perturbed and that late-generated pyramidal neurons fail to migrate past the previously generated ones. In addition, in BALB/c mice the mossy fibers which project from the granule cells of the dentate gyrus to area CA3c are distributed abnormally, forming an extensive intrapyramidal synaptic field in contrast to the normally occuring infrapyramidal synaptic field (Barber et al., '74, J. Comp. Neurol. 156: 417-434).

To determine the mode of inheritance of this strain difference, the distribution of mossy fibers was examined using the Timm's sulphide silver method in BALB/c x C57BL/6 F1 and F2 hybrids, in BALB/c and C57BL/6 mice which were fostered to females of the other strain before receiving their first meal, and in the CXB series of recombinant inbred strains (made using BALB/c and C57BL/6 as progenitor strains). The distribution of mossy fibers was classified as "BALB/c-like" if an intrapyramidal mossy fiber layer was present or as "B6-like" if an infrapyramidal mossy fiber layer was present.

In both male and female CB6F1 and B6CF1 hybrids the distribution of mossy fibers is BALB/c-like. In seven of nine F2 hybrids the distribution was BALB/c-like and in the remaining two B6-like. In the cross-fostered mice the pattern was always the same as normally raised mice of the same genotype. Of the recombinant inbred strains, five (CXBD, CXBG, CXBH, CXBI and CXBK) had BALB/c-like mossy fiber distributions and two (CXBE and CXBJ) had a B6-like distribution. These results are consistent with inheritance by means of a single autosomal gene. The degree of dominance of the gene and its linkage and the possible existence of modifying genes are under investigation.

The fact that, in a single subdivision of the CNS, a single gene affects both the position attained by migrating neurons and the establishment of connectivity of a population of axons indicates that the BALB/c mouse may be a useful tool for future studies of the cell biology of neuronal migration and neuronal specificity.

Supported by NSF Grant BNS-8120050 and NIH Biomedical Research Support Grant 5S07RR05386.

241.7 LACK OF FUNCTIONAL mRNA CODING FOR A MICROTUBULE-ASSOCIATED PROTEIN CORRELATES WITH POOR PERFORMANCE OF RATS IN A BEHAVIORAL PARADIGM. P. Strocchi, G.S. Zubenko* and J.M. Gilbert. Mailman Research Center, McLean Hospital, Belmont, MA 02178 and Harvard Medical School, Boston, MA 02115.

The cerebral cortical cells of Sprague-Dawley albino rats contain polysomal mRNA which directs the synthesis of one or both of two proteins, denoted A and B by Strocchi et al (1981), which co-purify with tubulin. The A and B proteins have apparent molecular weights of 54,000 daltons, but differ in their isoelectric points. The translation products synthesized from polysomal mRNA prepared from 153 individual cortices were subjected to two-dimensional polyacrylamide gel electrophoresis and fluorography. Three classes emerged with respect to these proteins: A, B, and AB. These qualitative differences in translation products reflect the presence of specific templates and not the inclusion of specific inhibitors of translation or contamination by an AB interconversion system since mixtures of polysomal mRNA that separately direct the synthesis of only one of these proteins direct the synthesis of both of these proteins in vitro. The similarity of these proteins along with the absence of rats whose cortices lack polysomal mRNA for both A and B (A^-B^-) are findings that are consistent with the hypothesis that the structural genes for these proteins are allelic.

As a serendipitous observation, we noticed that rats that performed poorly in a novel escape-avoidance paradigm tended to lack the functional RNA from cortical polysomes that encoded the A protein. Performance was measured by the length of time required for an animal to escape into the preferred dark compartment, a parameter called light-dark transition latency or LDTL (Jarvik and Kopp, 1967; Heinze, 1974; Strocchi et al, 1977). With a two-sample Kurskal-Wallis test, no significant difference ($p = 0.55$) could be demonstrated between the A and AB phenotypes. However, significant differences were observed between the A and B phenotypes ($p = 0.02$) and between the AB and B phenotypes ($p = 0.002$). The A and AB phenotypes are associated with a good performance and the B phenotype (A-deficient) with poor performance. This association does not result from a paradigm that induces a deficiency for protein A-encoding messenger RNA in the cortices of poor performers, for there is no increase in the relative frequency of animals bearing this phenotype in tested populations as compared to naive control populations. This deficiency may correspond to one of the genetic determinants previously demonstrated to affect this behavior in outbred strains of rats (Bignami, 1965; Broadhurst and Bignami, 1965; Wilcock and Fulker, 1973; Heinze, 1974; Wilcock et al, 1981; Hewitt et al, 1981). Supported by NIH grants MH36224, 5K02 70713 and The Marion Benton Trust.

241.8 IS THE β-NERVE GROWTH FACTOR GENE DEFECTIVE IN FAMILIAL DYSAUTONOMIA? X.O. Breakefield*[1], C.M. Castiglione*[1], L. Coussens*[2], F. Axelrod*[3] and A. Ullrich*[2] (SPON: D. Corey). [1]Dept. Human Genetics, Yale Univ. Sch. Med., New Haven CT 06510; [2]Genentech Inc., So. San Francisco CA 94080; [3]Dept. Pediatrics, N.Y. Univ. Med. Ctr., NYC 10016.

The availability of a cloned genomic probe for the human β-nerve growth factor (β-NGF) gene (Ullrich, Gray, Berman and Dull, in press) allows us to determine whether or not a mutation in this gene causes the inherited neurologic disease, familial dysautonomia. This disease is transmitted in an autosomal recessive manner and affects some 500 people world-wide, mostly of Ashkenazic Jewish descent. Symptoms include loss of sympathetic and sensory functions, e.g. poor control of digestion and blood pressure, insensitivity to pain and temperature, and lack of overflow tears and taste buds. Neuropathologic examination shows substantial loss of neurons in sympathetic, sensory and some parasympathetic ganglia even at early ages; the central nervous system is apparently unaffected (Pearson, Johnson and Brandeis, 1983, Devel. Biol. 96:32). Many of the pathologic features of this disease resemble those of rodents treated with antibodies to β-NGF in utero (Gorin and Johnson, 1980, Devel. Biol. 80:313) or post-natally (Aloe, Cozzari, Calissano and Levi-Montalcini, 1981, Nature 291: 413). Work by Ullrich and co-workers has shown that a single locus for β-NGF is present in the human haploid genome and that it codes for a protein with 90% homology to mouse β-NGF. We have used cloned fragments of this gene as probes to determine whether variations in DNA sequence occur in or near the β-NGF gene in families with dysautonomia. Variations in sequence are identified as differences in the length of DNA fragments that hybridize to these probes following digestion of genomic DNA with restriction endonucleases. Over 20 restriction endonucleases that cut the DNA at specific 4 and 6 base pair sequences have been tested. Two endonucleases have revealed variations in fragment length among individuals, and these variations are inherited as co-dominant alleles in families. These variations appear to be due to the presence or absence of specific endonuclease sites in non-coding regions of the β-NGF gene, and have no apparent relationship to the mutation causing the disease. These fragment length variations do serve, however, to mark both alleles of the β-NGF locus in some parents. Studies are underway to establish whether there is co-inheritance of specific β-NGF alleles from the parents and the disease state in six families. This analysis will exclude or implicate a lesion in the structural gene for β-NGF, including exon, intron and flanking sequences, as the cause of familial dysautonomia.

241.9 PNMT ACTIVITY IN THE RAT: CO-INHERITANCE OF ADRENOMEDULLARY AND REGIONAL BRAIN ENZYME? J.M. Stolk* R.B. Guchhait, G. Vantini*, B.D. Perry, D.C. U'Prichard and R.C. Elston* Maryland Psychiatric Research Center, Univ. of Maryland Sch. Med., Baltimore, MD 21228; Department of Pharmacology, Northwestern Medical School, Chicago, IL 60611; Department of Biometry, Louisiana State University Med. Sch., New Orleans, LA 70112.

PNMT, the enzyme that converts norepinephrine to epinephrine in adrenergic cells, is found in the adrenal medulla as well as in limited populations of neurons arising from the brain stem and distributing to spinal cord, medulla-pons, and hypothalamus. The adrenal enzyme is inherited as an autosomal co-dominant trait at a single locus in the mouse, presumably due to a regulatory gene (Ciaranello & Axelrod, JBC 248: 5616, 1973). We recently observed greater than 5-fold differences in adrenal PNMT activity within inbred rat strains; we employed the 2 strains having maximally different PNMT activity to assess inheritance, the type of gene or genes governing the adrenal enzyme, and possible co-inheritance of adrenal and brain PNMT activity. Inheritance was assessed in a F344 by Buf Mendelian cross. Enzyme activity in adult offspring yielded no evidence for sex linkage (in reciprocal cross hybrid males); inheritance is best accounted for by a single major autosomal gene superimposed upon a polygenic background. Detailed studies in the 2 parent strains strongly suggest that a structural gene is involved. The Buf enzyme is highly susceptible to thermal inactivation while the F344 enzyme is resistant; structural variants in the enzyme, perhaps, are associated with observed differences in affinity for the co-substrate, S-adenosyl-L-methionine (SAMe), although the strain with the more thermolabile enzyme has the higher affinity for SAMe (K_m= 3 μM in Buf, and 7 μM in F344). Analyses of medulla-pons and hypothalamus PNMT in F344, Buf and F_1 male offspring reflect directly the adrenal enzyme activity. Correlation coefficients of 0.786 and 0.920 are obtained for adrenal with medulla-pons or with hypothalamus, respectively. Initial evaluation of the F_2 generation similarly indicates a strong, positive correlation between adrenal and hypothalamus PNMT activity (R=0.874). These data are consistent with co-inheritance of adrenal and brain PNMT activity in the rat, and suggest that a single major structural gene is specifically involved. That the co-inheritance of adrenal and brain PNMT has functional implications is suggested by the inverse relationship between enzyme activity and α-adrenergic receptor density only in peripheral and central tissues with direct access to the PNMT product, epinephrine. (Supported by MH 32842, NS 15595, DA 02763, RSDA MH 00018 and NRSA MH 08834).

DEVELOPMENT OF INVERTEBRATES II

242.1 EVOLUTIONARY ORIGINS OF GIANT AXONS IN DROSOPHILA (DIPTERA). David G. King. Dept. of Anatomy and Dept. of Zoology, Southern Illinois University, Carbondale, IL 62901

Giant axons, like other highly differentiated neurons, pose several problems for neurobiologists: What is the adaptive value (if any) for such unique neuronal characteristics as great axonal diameter? What developmental mechanisms regulate specific features like axon size? How are the properties of specialized neurons encoded genetically? In evolutionary perspective, how can genetic variation in combination with natural selection mould development to yield individual adaptation in specific neuronal pathways? Comparative study of homologous neurons in related species may help elucidate such questions, by revealing both the ontogenetic constraints and the degrees of freedom available for evolutionary modification of specific neuronal form and function.

A pair of giant axons have long been known in Drosophila (Power '48, J.Comp.Neurol. 88:347). Recently similar axons have been reported in several other flies (King & Valentino in press, J.Comp.Neurol.; Strausfeld & Bassemir in press, J.Neurocytol.) These axons are well suited for phylogenetic investigation. Not only are they accessible to a variety of techniques, but they are found in a large and diverse group of animals that are already popular for developmental, genetic and evolutionary study. These giant axons appear to be adaptive; they provide increased speed and/or reliability for impulse conduction in a neural pathway which mediates a startle response. In the present study, giant axons were found in occasional nematocerans ("primitive" flies) and lower brachycerans, but these axons differ in course and position within the cervical connective from those previously reported in muscoid flies. Thus giant axons apparently do not form a primitive component of the dipteran system, but have evolved independently in different suborders. Giant axons similar to those in Drosophila were found only in the Cyclorrhapha (the most "advanced" of the three dipteran suborders) and in a brachyceran group closely related to the Cyclorrhapha. But giant axons are not present in all higher flies. The apparent homology among such axons when they do occur suggests that they have either evolved several times from homologous precursors or else been modified or lost from several taxa following their first appearance.

Whether giant axons can arise by evolutionary modification of the size of certain axons independently from others, or whether their exceptional size reflects one extreme of a size distribution whose evolutionary modification affects many other axons as well, remains to be determined. That many different neurons are characterized by specific axon size is clear (e.g., King & Tanouye in press, J.Exp.Biol.). To what extent such specificity is adaptive and/or subject to individual genetic modification is still unknown.

242.2 COMPUTER-AIDED MORPHOMETRY OF DEVELOPING AND MATURE ANTENNAL LOBES IN THE MOTH *MANDUCA SEXTA*. A.M. Schneiderman, J.G. Hildebrand and J.J. Jacobs*. Dept. of Biol. Sci., Columbia Univ., NY, NY 10027.

The antennal lobes (ALs) in the brain of *Manduca sexta* are sexually dimorphic. In addition to "ordinary" glomeruli, which are present in both male and female ALs, the male AL exclusively contains a large macroglomerular complex (MGC). The primary or only site for processing sensory information about female sex pheromone, the MGC contains dendritic arborizations of male-specific AL neurons [*Proc. Roy. Soc. Lond. B213*:249 (1981)] and arises during adult development only in ALs contacted by ingrowing male antennal sensory axons [*Nature 298*:844 (1982)]. As part of our studies of the functional organization and development of ALs and especially of their sexually dimorphic components, we are enumerating the glomeruli and neurons in male and female ALs and tracing the development of the MGC in normal male, and surgically produced gynandromorphic female, ALs [*Nature, Ibid.*].

We use the CARTOS system [*Annu. Rev. Biophys. Bioeng. 18*:323 (1979)] to visualize in 3-space the outlines and anatomical positions, volumes, and number of glomeruli and neuronal somata in ALs. Profiles are aligned and traced from serial sections stained by Bodian's silver-Protargol method, and with the aid of computer programs for quantitative manipulation of positional data, the number and volumes of glomeruli and the number of neuronal somata in each AL are estimated. Observations to date suggest that the number of "ordinary" glomeruli in male and female ALs may vary. Three normal female ALs exhibited 57, 57 and 61 glomeruli; 3 normal male ALs yielded 59, 61 and 61 (plus MGC); and 1 gynandromorphic female AL innervated by grafted male antennal afferents had 57 ordinary glomeruli plus an MGC. Such findings do not yet permit a decisive comparison between the populations of glomeruli in male and female ALs, but our evidence argues that the MGC arises independently of other glomeruli. We find that the nascent MGC can be identified, separate from the ordinary glomeruli, as early as day 5 of the 18-day course of adult development -- the second day (out of *ca.* 8) of ingrowth of antennal afferents and the stage at which glomeruli are first discernible in the AL neuropil [*Soc. Neurosci. Abstr. 7*:3 (1981)]. With the aid of the cell-counting program CELLS, we have also begun to count neuronal somata in normal and gynandromorphic male and female ALs. Our goal is to ascertain whether the number of AL neurons in males is greater than that in females as a preliminary test of the hypothesis that male antennal sensory axons "rescue" a population of AL neurons produced in both male and female ALs but destined ordinarily to die in normal females. Our findings from this effort will be reported.

(Supported by NSF grant BNS 80-13511 and NIH grant AI-17711, and by NIH grant RR-00442-14 to the Facility for Computer Graphics in the Columbia Univ. Dept. of Biol. Sci.)

242.3 SEXUALLY DIMORPHIC EXPRESSION OF POLYPEPTIDES IN ANTENNAL SENSORY NEURONS OF *MANDUCA SEXTA*. T.G. Kingan and J.G. Hildebrand. Dept. of Biol. Sci., Columbia Univ New York, NY 10027.

In the moth *Manduca sexta*, the neuropil of the male antennal lobes (ALs) contains a macroglomerular complex (MGC), which is not present in the female AL and receives primary-afferent inputs from the male-specific, pheromone-detecting neurons in the male antenna [Matsumoto and Hildebrand, *Proc. Roy. Soc. Lond. B213*:249 (1981)]. Experiments involving surgical production of chronically deantennated and gynandromorphic ALs have shown that the development of the MGC is controlled by the male sensory axons [Schneiderman, Matsumoto and Hildebrand, *Nature 298*:844 (1982)]. Because an MGC develops only in an AL directly contacted by ingrowing male antennal sensory axons, we hypothesize that the male fibers possess molecular "factor(s)" possibly polypeptides -- responsible for inducing or permitting the development of the MGC and associated male-specific AL neurons. We have begun to search for sexually dimorphic polypeptides (PPs) to be candidates for such a role.

To compare the patterns of PPs in the afferent axons, we label them *in vivo* with [^{35}S]methionine and subsequently analyze the labeled PPs by gel electrophoresis. We inject [^{35}S]met into the antennal flagellum 20 mm from its base to expose a population of sensory neurons to radiolabeled amino acid; extract the PP fraction from the intracranial segment of the antennal nerve (AN) and AL; separate the PPs by 2-dimensional gel electrophoresis; and visualize them by silver-staining and fluorography. Among the PPs we detect in extracts made 24 hr after injection is one with an apparent molecular weight of 24 kilodaltons (24KD-PP), which is greatly enriched in male ANs and ALs. This 24KD-PP is still detectable in AN extracts 3 days after injection of [^{35}S]met, when many more slowly transported PPs first appear in the intracranial AN and AL, and it appears to be produced or transported only between day 6 and at most day 16 of the 18-day period of adult development. Other experiments suggest that this 24KD-PP is not synthesized in the AL, optic lobes, or protocerebrum.

Although silver-staining alone does not reliably reveal the 24KD-PP, this procedure does demonstrate reproducible differences between the apparent levels of 4 51KD-PPs (with slightly different mobilities in the charge-dependent first dimension of the gels) in developing male and female ALs. In contrast to the 24KD-PP, the 51KD-PPs are not among the most rapidly transported PPs in the AN; they appear in the intracranial AN only about 3 days after *in vivo* labeling with [^{35}S]met. The sexual differences in the 51KD-PPs can be detected by silver-staining between days 6 and 14 of adult development. By day 18, however, the sexually dimorphic pattern of the 51KD-PPs has given way to a distribution resembling that in the developing male AN. (Supported by grants NSF BNS 80-13511 and NIH AI-17711 and an NINCDS Postdoctoral Fellowship to TGK).

242.4 POST-EMBRYONIC DEVELOPMENT OF GRASSHOPPER OVIPOSITORS AND THE OVIPOSITION MOTOR PROGRAM. Karen J. Thompson and Eric Schabtach.* Dept. of Biology, Univ. of Oregon, Eugene, OR 97403.

Mature female grasshoppers excavate chambers for the burial of egg-pods by rhythmical movements of specialized appendages (ovipositors) located on the abdomen tip. A central pattern generator (CPG) in the terminal abdominal ganglion drives these movements.

The periodic ovipositor muscle contractions are normally seen only in sexually mature females; however, the CPG underlying them can be activated as early as the fourth (penultimate) larval instar. Activation of the CPG is achieved by severing the rostral neural connections to the terminal abdominal ganglion. Neither in larval animals nor in immature adults does the recorded electromyographic pattern lead to mechanical activity in the muscles. Additionally, there appears to be suppression of the motor output in immature adults, since the electromyographic activity recorded from these animals is significantly reduced by comparison to both the mature and larval cases

Scanning electron microscopy was used to examine the structural development of the ovipositor. The ovipositor consists of paired appendages which are outgrowths of the posterior margins of the eighth and ninth segments. These appendages are present as rudimentary buds in the first larval instar. In successive instars they are enlarged, modified, and the sternal plate between them reduced such that they become two pairs of shovel-shaped structures aligned dorso-ventrally at the final moult to adulthood. Further development occurs in the immature adult. This includes substantial enlargement of ovipositor cuticle and muscle. The ovipositor is also heavily sclerotized during this time.

In conclusion, the structure of the ovipositor develops throughout larval and immature adult life. The functional state is achieved by the onset of sexual maturity. In contrast, the CPG for oviposition digging is fully functional in the larval stage, long before it is ever used by the animal.

Supported by GMO 7257 to K.T. and BNS 79/23786 to G. Hoyle.

242.5 THE EFFECTS OF HYPOINNERVATION UPON THE FREQUENCIES OF FLY PHOTORECEPTOR SYNAPSES. A.Fröhlich & I. A. Meinertzhagen, Life Sciences Centre, Dalhousie University, Halifax, N.S. Canada

In the first optic neuropile, or lamina ganglionaris, of the fly's visual system photoreceptor terminals form tetrad synapses with precisely controlled frequencies. The receptors are grouped into units (cartridges) such that normally six receptor terminals synapse upon two monopolar cells and upon α-processes of amacrine cells. Where dorsal and ventral halves of the lamina meet, at the so-called equator, a zone of naturally produced hyperinnervation occurs, cartridges having seven or eight presynaptic receptors instead of six. The number of postsynaptic cells remains constant. Previous observations (Nicol and Meinertzhagen, 1982) revealed that in the case of this hyperinnervation the synaptic frequency per cartridge does not stay constant, as it should if the number of synapses were controlled postsynaptically. Instead, the synaptic population per cartridge rises. Control of synapse frequency during hyperinnervation was therefore assumed to be largely presynaptic, with the postsynaptic cells accommodating to the increased synaptic load.

We have studied the reciprocal hypoinnervation normally occurring at the edge of the lamina at cartridges with two, three, four or five receptor terminals. Thus the range of observations is now extended from an innervation ratio of 8:6 (previously reported for the equator) to one of 2:6, at the edge of the lamina. Receptor terminals are smooth, cylindrical and orientated in parallel in the lamina. The number of their synapses and the perimeter of their transverse profiles are both readily sampled quantitatively in single-section EM.

As the number of receptor terminals per cartridge decreases, the number of synapses per terminal increases. This increase gets larger as the hypoinnervation becomes more severe. Thus there is a marked, though not complete, compensation for the decrease in the number of innervating receptor terminals. This suggests that in hypoinnervated cartridges the postsynaptic influence upon synaptic frequency is stronger than previously anticipated. The predominant influence upon synaptic frequency seems to shift with the innervation ratio, from largely presynaptic (in hyperinnervated cartridges) to largely postsynaptic (in hypoinnervated cartridges). The perimeters of receptor terminal profiles co-vary with the number of their synapses. Thus terminals of hypoinnervated cartridges are large and bear more synapses than their smaller counterparts in hyperinnervated cartridges. Consequently the mean presynaptic membrane perimeter per synaptic profile remains roughly constant despite variations in innervation ratio and subsequent changes in synaptic population size.

Supported by grants EY-03592 from NIH and A-0065 from NSERC.

242.6 **DEVELOPMENT OF SENSORY HAIRS IN JUVENILES OF THE CHITON MOPALIA MUSCOSA.** **E.M. Leise* (SPON: B. Mulloney). Dept. of Zool., Univ. of Wash., Seattle, WA 98195**

The mantle epidermis surrounding the shell plates of Mopalia muscosa is covered by a cuticle and secretes tapered chitinous hairs and calcareous spicules. Hairs, up to 5 mm long and 400 μm wide, extend through and beyond the dorsal cuticle while spicules of various sizes lie within the dorsal, marginal and ventral cuticles. Stalked nodules, extensions of multicellular epidermal papillae, occur separately within the dorsal cuticle, within the dorsal hairs and at the bases of marginal and ventral spicules. Papillae that produce hairs were found to be innervated. Dendrites of putative sensory neurons occur in the stalked nodules; all nodules are proposed to be mechanosensory.

The integuments of larval, juvenile and adult M. muscosa are dissimilar. I examined the integuments of juveniles raised in the laboratory for one year to determine the sequence of events that occurs as the epidermis and particularly its sensory organs develop adult characteristics. At metamorphosis larvae secrete a cuticle over the dorsum, extrude small (3 μm by 9 μm) spicules into the cuticle and secrete the shell plates and small (2 μm wide and 15 μm long) chitinous hairs. Adult and juvenile spicules are discrete structures; the first juvenile spicules have no associated stalked nodules and are not innervated. Juvenile hairs become the tips of adult hairs. Each hair is initially secreted by one trichogenous cell. Within two days after metamorphosis the dendrite from a hair's first sensory cell protrudes into the adjacent trichogenous cell. This trichogenous cell will become part of the supporting sheath of a nodule. Each adult hair contains a row of mesial spicules and each spicule surmounts a stalked nodule. As a hair grows it develops more spicules and stalked nodules and hence, more sensory cells. Nodules in the hair develop sequentially and continue to elongate as the hair grows. Hairs do not develop all of their adult features until they are at least 300 μm long, at about one year of age in laboratory animals.

242.7 TRANSPLANTATION OF CRICKET SENSORY NEURONS TO ECTOPIC SITES REVEALS RULES FOR ASSEMBLING AFFERENT PROJECTIONS. S.E. Johnson* and R.K. Murphey (SPON: R. Oesterreich) Dept. of Biology, SUNY Albany, Albany, New York, 12222

Insect sensory systems are favorable for the study of the development of neural patterns because of the ease with which the sensory neurons can be manipulated, either surgically or genetically. We have discovered that in each segment an important step in the differentiation of sensory neurons commits these cells to arborize in one of two target areas. In the cercal system this step commits cells to arborize in one of two regions in the terminal abdominal ganglion. These two regions process information of different sensory modalities (Murphey, this vol.).

We have previously shown that when cerci were transplanted to the thorax, this dichotomy was retained: bristle hair sensory neurons arborized in a slab of neuropil on the ventral surface of the ganglion while filiform hairs arborized in an adjacent, more dorsal region. We have now looked at the normal projection pattern of leg bristle hair sensory neurons to the mesothoracic ganglion. Cobalt fills of single bristle afferents on the leg revealed that they arborize only in the most ventral neuropil area coextensive with the ventral association center (VAC). Thus bristles on the leg and on the cercus both choose to arborize in the same region of thoracic neuropil. Within the bristle hair target region we found that the morphology of single leg afferents reflects circumferential position of the receptors on the leg. Therefore the spatial organization of receptors on the limb is reflected in the CNS.

To further probe the rules for ordering sensory inputs to the CNS, we transplanted mesothoracic legs to the abdomen, forcing the leg sensory axons to regenerate into the terminal abdominal ganglion. The leg bristle hair neurons were able to discriminate between the two neuropil regions in the foreign ganglion: the bristle hair axons grow under the cercal glomerulus to arborize in the bristle-hair neuropil area. The leg afferents also maintain many aspects of their normal orderly projection pattern in this foreign ganglion.

In summary, sensory neurons in any region of the cricket body are of two types: a "bristle" type which is multiply innervated and arborizes in one area of neuropil. A second type includes all other receptors on or in the appendage which arborize just dorsal to the bristle hair area. A search of the literature reveals that this dichotomy appears to exist in other insects as well. This finding is important because it greatly simplifies the decisions a sensory neuron growth cone must make in order to find its synaptic partners. Supported by NIH Grant #NS 15571 to R.K.M.

DEVELOPMENT AND PLASTICITY: TROPHIC AGENTS II

243.1 BRAIN EXTRACT INCREASES LEVELS OF A_{12} ACETYLCHOLINESTERASE (AChE) IN CULTURES OF CHICK SKELETAL MUSCLE MAINTAINED IN HORSE SERUM-FREE MEDIUM. J.S. Addis* (SPON: N.L. Hayes). Dept of Anatomy, Univ. of Mississippi Med. Ctr., Jackson MS 39216.

It was recently reported that the appearance of the A_{12} form of AChE could be induced in cultures of chick skeletal muscle by removing horse serum (HS) from the medium (Bulger et al., Dev. Neurosci. 5:474, 1982). Since nerve extract has been observed to induce this form of AChE in chick skeletal muscle cultured in the presence of HS (Popiela et al., Soc. Neurosci. Abs. 8:195, 1982), it was of interest to determine what effect a nerve extract would have on the appearance of A_{12} AChE in muscle cultured in the absence of HS. Cultures, prepared by mechanical dissociation from the leg muscles of 11-day chick embryos, were maintained for 6 or 8 days in Dulbecco's MEM supplemented with 2% chick embryo extract/50 U ml^{-1} Penicillin/ 50 ug ml^{-1} Streptomycin/10% HS. Medium lacking HS then replaced the HS-containing medium for 4 or 2 days. Mouse brain extract (BE) was introduced into the HS-free medium at a concentration of 250 or 500 ug ml^{-1} after 8 days. At the same time, control cultures received medium to which bovine serum albumin had been added. After 10 days the cultures were extracted in 0.1 M sodium phosphate buffer, pH 7.0/0.15 M NaCl/0.25 mM EDTA/0.5% Triton X-100/0.1 mg ml^{-1} Bacitracin, and the molecular forms of AChE separated in 5-20% sucrose gradients.

In cultures maintained in HS-free medium for days 9-10, BE induced increases in both A_{12} activity (~3.5X) and in the ratio of A_{12} activity to total AChE activity (~2.3X). The latter value rose from ~3.1% in control cultures to ~7.3% in BE-treated cultures. Total AChE also increased in BE-treated cultures (~1.5X). In cultures maintained in HS-free medium for days 7-10, BE likewise induced increases in A_{12} activity (~2.3X) and in the ratio of A_{12} to total activity (~2X). Under these conditions, the ratio increased from ~7.5% to ~15%. Total activity in BE-treated cultures maintained from 7-10 days in HS-free medium was slightly less (~0.9X) than that in control cultures.

These results indicate that the addition of BE for a period as short as 2 days to muscle cultured in HS-free medium enhances both the absolute levels of A_{12} activity and the proportion of A_{12} to total AChE activity. The results moreover argue against the possibility that the induced appearance of A_{12} AChE by nerve extract in HS-containing medium is due to interaction between constituents of the extract and the serum.

(Supported by N.I.H. Biomedical Research Support Grant 5 S07 RR05386.)

243.2 TROPHIC INFLUENCE OF EPIDERMAL GROWTH FACTOR, FIBROBLASTIC GROWTH FACTOR AND INSULIN ON HUMAN MUSCLE DEVELOPMENT IN TISSUE CULTURE. G. Gallez-Hawkins* and V. Askanas. USC Neuromuscular Ct., Dept. of Neurology, Univ. So. Calif. Sch. of Med., Los Angeles, CA 90017.

Until now culturing of adult human muscle has required addition of both 10-15% fetal calf serum (FCS) and 2-5% chick embryo extract (CEE) to the medium in order to obtain satisfactory growth and maturation. Attempting to establish a more defined medium, which would eventually allow elimination of CEE and FCS and lead to better maturation of cultured adult human muscle, we introduced, per ml culture medium, 10 ng of epidermal growth factor (EGF), 50 ng fibroblast growth factor (FGF), and 10 ug insulin (I), separately and in combination with each other, with and without CEE and/or FCS. Cultures were established according to our standard conditions (Askanas, V. & Engel, W.K., Neurology 25:58, 1975), from 1 mm^3 explants or dissociated single cells. Living cultures were evaluated every day by phase-contrast inverted microscopy. Between 10-25 day of growth they were harvested for creatine kinase (CK) activity estimation. 11 experiments were established and 2-4 cultures in each experiment served for a given combination. Not all conditions were evaluated in each experiment. The mean and P values are each based on at least 8 experiments. a), b) and c) indicate three different culture conditions.

Morphology: a) omission of CEE resulted in much less myogenic appearance and greatly delayed myoblasts fusion. b) replacement of CEE with the triple combination of EGF, FGF and I resulted in decreased amount of fibroblasts, accelerated fusion, and the presence of well-developed, thick myotubes. c) in the regular condition (with CEE, but not I, EGF and FGF), fusion was 2-3 days later, cultures were more fibroblastic, and myotubes less mature than in (b). Biochemistry: Combined addition of I, EGF and FGF (condition b), resulted in 6.7 fold ($P < 0.005$) higher CK as compared to sister cultures with the addition of CEE but no factors (condition c), and 15.7 fold ($P < 0.001$) increase of CK as compared to sister cultures without CEE and no factors (condition a). There was 50% less CK activity in a) compared with c). Addition of I and FGF resulted in 2.8 fold, and I and EGF in 3.7 fold higher CK as compared to c, but statistical significance of these data are uncertain. Those double-factor additions, however, did give statistically significant increase of CK activity compared with condition a). Addition of I, EGF and FGF alone did not result in substantial increase of CK activity as compared to c). Total depletion of FCS resulted in no growth in spite of addition of I + EGF + FGF, but reduction of FCS to 2-5% was possible.

These studies: 1) provide a more defined (CEE free, FCS reduced) new medium for culturing of adult human muscle, 2) demonstrate synergistic positive influences of I, EGF and FGF on human muscle development and maturation in culture, and 3) provide a system in which selective lack of influence of those hormones individually on abnormal muscle might be demonstrable.

243.3 BENEFICIAL INFLUENCE OF INSULIN ON CHOLINERGIC PROPERTIES AND ENERGY METABOLISM OF RAT CULTURED VENTRAL SPINAL CORD NEURONS (CVSCNs) F. Moriwaka*, V. Askanas, W. K. Engel. USC Neuromuscular Ctr., Dept. of Neurology, Univ. So. Calif. Sch. of Med., Los Angeles, CA 90017.

We have previously demonstrated that activity of creatine kinase (CK) was high in rat CVSCNs, which contain lower motor neurons, and that the CK activity parallels the activity of cholineacetyltransferase (ChAT) in a given culture, as well as in the ventral part of spinal cord of fetal and adult rats in vivo (Schmidt-Achert, K.M., et al., Neurology 33:143, 1983). To the contrary, CK activity is practically undetectable in rat cultured schwann cells and very low in cultured skin fibroblasts. Therefore both ChAT and CK activities are measured in our laboratory when investigating the influence of various factors on CVSCNs. Cultures of the ventral part of spinal cord were established from 15-16 day old rat fetuses, according to the technique developed in this laboratory (Askanas, V. et al., Neurology 31, 1196, 1981). Cultures were established with 1 mm^3 explants; ventral regions of one entire spinal cord were placed in one 35 mm collagen-coated petri dish. 5 experiments were performed and in each experiment 4 culture dishes served for a given experimental condition. 10 ug and 25 ug of insulin (Sigma) were incorporated into culture medium from the time of initiation of the culture. During the first 5-8 days of growth, the culture medium consisted of Dubelcco's Modified Eagle's Medium (DMEM) with 400 mg% glucose and no glutamine, 10% fetal calf serum (FCS), and insulin. During this time non-neuronal cells were significantly reduced by an initial 3 days of antimitotic treatment. After antimitotic treatment was accomplished, FCS was eliminated from the medium. Then, control CVSCNs were cultured in DMEM alone, while experimental sister CVCSNs received DMEM plus various concentrations of insulin for 10-15 days. Elimination of FCS from the medium provided a suboptimal condition for control cultures and allowed more clear evaluation of insulin action on CVCSNs, since other hormonal influences were eliminated. ChAT, CK, acetylcholinesterase and total protein were measured. Treatment with insulin resulted morphologically (by phase-contrast examination) in better survival of neuronal cells and richer, more abundant neuronal processes. ChAT activity in insulin-treated cultures was increased 7.8 fold ($P = 0.02$) as compared to control cultures, and CK activity increased 4.7 fold ($P = 0.01$). Protein increased 2.3 fold ($P = 0.02$) and cholinesterase activity increased 2.2 fold ($P > 0.05$). Those results were not insulin dose related in the range studied. The results indicate a) definite beneficial influence of insulin on neuronal survival and on cholinergic and energy metabolism properties of rat CVSCNs, presumably reflecting properties mainly of lower motoneurons. The insulin effect on CVSCNs is probably trophic and receptor mediated. The observed increase of protein and cholinesterase with insulin treatment could be related to non-neuronal (as well as neuronal cells), on which the insulin might exert trophic and some mitogenic action in spite of antimitotic treatment.

243.4 TROPHIC EFFECTS OF MAMMALIAN NERVE EXTRACT IN VIVO ARE NOT DUE TO TRANSFERRIN. H.L. Davis and E.A. Heinicke*. Departments of Biochemistry and Anatomy, The University of Western Ontario, London, Canada, N6A 5C1.

Atrophy in a denervated muscle results from the disuse caused by paralysis of the muscle, and from the loss of special neurotrophic substances. Proteins extracted from rats' sciatic nerves have been shown to prevent the non-disuse atrophy of rats' extensor digitorum longus (EDL) muscles denervated for 7 days, when administered by daily intramuscular injections. Trophic influences of extracts of peripheral nerve have also been demonstrated on embryonic chick muscle in vitro. The trophic factor from chicken sciatic nerve has been purified and was found to be closely related in structure to transferrin (Markelonis et al., 1982:J. Neurochem. 39,315-320). The present investigation was undertaken to determine whether the trophic properties of mammalian peripheral nerve extract on denervated rats' muscles in vivo are due to the presence of serum transferrin in the extract.

Rats' EDL muscles denervated for 7 days and injected daily with one of several doses of iron-conjugated transferrin purified from rat serum exhibited a rate of atrophy equivalent to that in denervated muscles that either were not treated or were injected with buffered saline. Atrophy was assessed by measurement of wet weight and cross-sectional areas of muscle fibers in both denervated and normal contralateral EDL muscles. Data were expressed as percentages of normal control values.

Denervated EDL muscles injected with crude extract of rats' sciatic nerves had significantly smaller decreases in wet weight and areas of type IIB fibers (in sections stained for ATPase) than in untreated or saline injected controls. Transferrin was removed from the crude extract by immunoaffinity chromatography. The resin was produced by coupling Ig fraction of anti-rat transferrin to activated CH-Sepharose 4B. The specificity and capacity of the coupled ligand was tested by passing pure rat transferrin or rat serum through the column and subsequently examining eluted and desorbed fractions with polyacrylamide gel electrophoresis. Removal of transferrin from crude rat nerve extract did not diminish its ameliorative effects on denervated muscle.

Thus the trophic action of mammalian nerve extract on denervated rats' muscles in vivo is not due to the presence of serum transferrin in the extract.

Supported by the Muscular Dystrophy Association of Canada and the Medical Research Council of Canada.

243.5 PROTEINS FROM CONDITIONED MEDIA BIND TO NEURONS. Leonard M. Gralnik*, John N. Barrett, and David L. Wilson (SPON: Alan H. Lockwood). Department of Physiology and Biophysics, Univ. of Miami Sch. of Medicine, Miami, FL 33101

Skeletal muscle cells and lung fibroblast cells obtained from fetal rats and grown in tissue culture release a reproducible array of proteins into their media. Labeled, conditioned medium was prepared from muscle or lung cultures by replacing normal growth medium with serum-free medium containing ^{35}S-methionine. The medium was collected and analyzed by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE). The labeled, released proteins are a small subset (less than 10%) of the total cellular proteins seen in homogenates analyzed by 2-D PAGE.

Unlabeled spinal cord cells from fetal rats were incubated with the labeled, conditioned medium from lung or muscle cultures. They were then washed extensively, and analyzed by 2-D PAGE to determine which proteins released by lung or muscle cells had bound to the spinal cord cells. A small number of proteins from medium conditioned by lung or muscle cultures (less than 10% of the proteins in the medium) bind to spinal cord cells (as well as to other CNS neurons).

Media conditioned by lung or muscle cells were chosen for this study because they are known to possess neurite-outgrowth promoting activity for spinal cord slices in vitro (Dribin and Barrett, Devel. Bio., 74:184, 1980). The binding experiments were carried out to search for the proteins eliciting the neurite outgrowth, since proteins that have trophic activity for spinal cord neurons should bind to the neurons. We acknowledge support from the National Parkinsons Foundation, from NSF grant BNS 81-17817, and from NIH grants NS 12207 and NIGMS 5T32GM07332.

243.6 THE NEURITE-PROMOTING EFFECT OF FIBROBLAST GROWTH FACTOR ON PC12 CELLS. Akifumi Togari,*Dole Baker,*Geneva Dickens*and Gordon Guroff. Section on Growth Factors, NICHD, NIH, Bethesda, MD 20205.

The PC12 clone of rat pheochromocytoma is the premiere model for the study of the actions of nerve growth factor. When these cells are treated with nanomolar quantities of the factor, they stop dividing, develop excitable membranes, grow neurites and will form synapses with appropriate muscle cells in culture. The wide-spread use of PC12 cells as a model has led to an interest in the various effectors acting on the cells. It has been shown that epidermal growth factor, TPA, and NECA, and cAMP derivatives have some effect on these cells. The actions of these other effectors are the same in some cases as those of nerve growth factor. However, none of these several other agents produces the single most characteristic action of nerve growth factor, namely, neurite outgrowth.

We report here that fibroblast growth factor, a partially purified material from either brain or pituitary, with mitogenic actions on a number of cell types, produces neurite outgrowth in PC12 cells comparable to that seen with nerve growth factor. These neurites are evident within 24 hours and continue to grow for at least 72 hours. The processes are long and thin, and appear to terminate in growth cones. Process formation is maximal at 50 ng of fibroblast growth factor per ml; brain and pituitary fibroblast growth factors appear to be equivalent. This action is preceded by an action on the marker enzyme, ornithine decarboxylase and, in both actions, fibroblast growth factor seems to be additive with nerve growth factor. The addition of equivalent amounts of platelet-derived growth factor or T-cell growth factor are without effect on either neurite outgrowth or ornithine decarboxylase activity.

The data suggest that the relevance of PC12 as a model system should be considered in the light of the many effectors which have been shown to act on these cells.

243.7 A NEW NEUROTROPHIC FACTOR ISOLATED FROM MAMMALIAN BRAIN: IN VITRO STUDIES ON FETAL RAT RETINA. J. E. TURNER, Y. A. BARDE AND H. THOENEN. Department of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina, U.S.A. and Department of Neurochemistry, Max-Planck Institute for Psychiatry, F. R. Germany.

Tissue culture conditions have been defined where stimulation of neurite outgrowth from fetal rat retinal explants occurred only in the presence of a newly purified brain trophic factor (PF) or an intermediate active fraction (BE) from the brain extract purification procedure. Under these conditions, 20-day fetal retinal explants survive and continue to extend neurite for at least 3 weeks in the presence of BE. There was demonstrated in vitro an inverse relationship between increased developmental age and responsiveness to BE. There was also a significant relationship demonstrated between the increased length of time before BE was added to the culture medium and the inability of 20-day fetal explants to extend neurites onto the culture substrate. These results may imply that BE not only is required for stimulating neurite outgrowth from fetal rat retinal explants, but it may possess some survival value with respect to the neurite producing retinal neurons. This was indeed found to be the case when thick sections were evaluated by light microscopy for cell survival. After one week in culture, control explants showed extensive degeneration where BE treatment ensured cell survival and explant differentiation. Most importantly, we found that PF, like BE, was able to stimulate neurite outgrowth from fetal retinal explants. More specifically, a dose-response relationship was demonstrated in both the presence of increasing concentrations of either BE or PF with respect to the neurite growth index. The half-maximal response for BE was estimated to be between 5-10 µg/ml and for PF it was approximately 20 ng/ml. This makes a strong case for the presence of high affinity receptors on the neurite producing retinal cells. In addition, a dissociated fetal retinal in vitro system has been developed where cells survive in the presence of BE for periods of at least 2 weeks, but untreated controls do not survive beyond 2-3 days in culture. In conclusion, we have reported for the first time that a new brain derived trophic factor can stimulate neurite outgrowth and maintain cell survival from an appropriate target tissue. The fetal rat visual system represents an exciting new model for testing further the importance of PF in the CNS. We postulate that PF may be like NGF in the PNS with respect to its importance in the developing and regenerating mammalian CNS.

(Supported in part by NEI Grant EY04377)

243.8 EFFECTS OF AN EARLY NERVE GROWTH FACTOR INJECTION ON ACTIVITY AND LEARNING OF A TWO-WAY ACTIVE AVOIDANCE TASK IN RATS WITH EARLY VENTROMEDIAL HYPOTHALAMIC OR SEPTAL DAMAGE. F. Eclancher*, J. J. Ramirez* and D. G. Stein. Brain Res. Lab., Dept. Psychology, Clark Univ.,Worcester, MA 01610.

Ventromedial hypothalamic (VMH) lesions or septal (S) lesions performed in 7-day old Wistar rats result in severe and persistent behavioral changes such as higher emotional reactivity and enhanced (S) or impaired (VMH) performance on a 2-way active avoidance (AA) task (Eclancher and Karli, 1982). The present study was undertaken to determine whether the same early lesions performed in Sprague-Dawley rats would result in similar behavioral changes. Moreover, since nerve growth factor (NGF) has been shown to ameliorate the behavioral effects of damage in certain subcortical areas (Hart et al., Brain Res. Bull., 3:245-250, 1978; Lewis et al., Brain Res., 176:297-310, 1979), we also wanted to determine whether NGF treatment immediately after VMH or S damage would alleviate the effects of the lesions. After being subjected to VMH or S lesions followed by NGF or saline injections directly into the damaged area, the rats were tested on an open field apparatus and a 2-way active avoidance (AA) chamber every 10 days from 20-90 days of age.

In confirmation of Eclancher and Karli's (1982) earlier work, lesions of the VMH resulted in hypoactivity in the open field test and in an impairment in the acquisition of the 2-way AA task. Septal lesions, in contrast, produced hyperactive behavior and facilitated the acquisition of the 2-way AA task. The NGF treatment had limited beneficial effects in both brain-damaged groups. The analysis of the number of crossings on the AA task, for example, indicated that the performance of the VMH-NGF and S-NGF groups approached control levels; more specifically, the VMH-NGF group made more frequent crossings between chambers whereas the S-NGF group made fewer crossings. In the open field activity test, the septal rats with the NGF treatment made significantly fewer line crossings than either the rats with saline treatment alone or the septal rats with saline treatment.

In general, it appears that early treatment with NGF may have long-lasting behavioral and limited ameliorative effects.

Supported by NIA Grant #5 R01 AG00295 and United States Army Research and Development Command Contract #DAMD-82-C-2205 to D. G. Stein, and grants from CNRS and NATO to F. Eclancher.

243.9 GM1 GANGLIOSIDE TREATMENT ENHANCES BEHAVIORAL RECOVERY FOLLOWING BILATERAL CAUDATE NUCLEUS DAMAGE. B. A. Sabel, M. D. Slavin* and D. G. Stein. Brain Res. Lab., Dept. Psychology, Clark Univ., Worcester, MA 01610.

Gangliosides, sialic acid containing glycolipids, are involved in neuronal development and repair. Toffano et al. (Brain Res., 261:163, 1983) found that rats treated with gangliosides showed less behavioral impairment following nigro-striatal hemitransection. An elevation of various enzymes in the striatum was taken to indicate that central sprouting may have mediated the behavioral recovery. In the present experiment, we investigated the effect of ganglioside injections on learning a cognitive task in rats with bilateral caudate lesions.

Male Sprague-Dawley rats (95 days old, 390-515 g) received sham operations (group C, n=8), or bilateral caudate lesions with IP injections of the monosialoganglioside GM1 dissolved in Ringer's solution (group LG, n=7), or Ringer's solution alone (group L, n=8). GM1 (30 mg/kg) was injected daily for 14 days, starting on the day of surgery. On the 10th postoperative day, animals were trained to run to their non-preferred side in a 2-choice learning maze for 10 trials daily. After they learned to avoid or escape footshock without error for two consecutive days (criterion), the rats had to run to the opposite side. In this manner, the animals underwent a series of spatial reversals for 30 days.

Both lesion groups (LG and L) were significantly impaired on this behavioral task compared to sham operates (C). However, rats with lesions and GM1-treatment performed significantly better on this task than animals with lesion alone (group L). Specifically, group LG had more days with 9/10 correct responses ($F=6.65$, $p<.023$), fewer escape failures per reversal ($F=5.33$, $p<.04$), and took fewer days to reach criterion after the first reversal ($F=9.8$, $p<.008$).

These results provide further support for the hypothesis that behavioral recovery from brain trauma can be enhanced by ganglioside treatment. To test whether GM1 treatment has long-lasting effects on recovery from brain trauma, the animals will be retested. A subsequent histological analysis will be done to explore physiological mechanisms that may underlie the behavioral recovery.

This research was made possible by the generous supply of GM1 from Fidia Laboratories, Abano Terme, Italy, and a contract from the United States Army Research and Development Command #DAMD-82-C-2205.

243.10 NERVE GROWTH FACTOR STIMULATES PHOSPHOLIPID METHYLATION IN TARGET GANGLIONIC NEURONS INDEPENDENTLY FROM THE CYCLIC AMP AND Na^+,K^+-PUMP RESPONSES. S. D. Skaper and S. Varon. Dept. Biol., Sch. of Med., Univ. of Calif. San Diego, La Jolla, CA 92093.

Our recent studies on the mechanism of action of Nerve Growth Factor (NGF) on its target ganglionic neurons have revealed that very dramatic changes are imposed within minutes after NGF administration. One response is an activation in the presence of NGF of the Na^+,K^+-pump in the neuronal membrane of sympathetic and sensory ganglionic cells. In another response, chick embryo dorsal root ganglia (DRG) display a rapid and transient rise in their cyclic AMP content when presented with NGF. It was subsequently shown that the cyclic AMP and ionic responses occur independently of each other in the chick DRG. The finding by Pfenninger and Johnson (1981) that NGF leads to a very early, though transient increase in phospholipid methylation in growing neurites from rat sympathetic neurons encouraged the speculation that such a cell function could be implicated in both short-latency responses. To test this idea, suspensions of neurons prepared from embryonic day 12 (E12) chick sympathetic ganglia (SG) were incubated with [methyl-^{3}H]methionine in the absence of NGF. Presentation of NGF for different periods of time resulted in an approximate 3-fold stimulation of radioactivity incorporated into total phospholipid, followed by a rapid decline thereafter. Both the magnitude and time of the response were dependent upon the NGF concentration used. Incubation of E12 SG neurons with transmethylase inhibitors failed to prevent reactivation of the Na^+,K^+-pump in response to NGF administration. E16 SG neurons and E15 DRG neurons, which do not depend upon exogenous NGF for control of their Na^+,K^+-pump still show a stimulation of phospholipid methylation when challenged with NGF. Blockage of the pump with ouabain also fails to prevent a methylation response. Thus, the pump and methylation responses to NGF occur independently of each other. NGF is also capable of eliciting a methylation response in intact E8 chick DRG, in addition to the transient rise in cyclic AMP content. For a given concentration of NGF, the peak of phospholipid methylation preceeds the peak of cyclic AMP. Methylation inhibitors prevent the methylation response, but not the cyclic AMP one. Dibutyryl cyclic AMP, which is able to cause a cyclic AMP elevation similar to NGF failed to produce any stimulation of phospholipid methylation. These data thus indicate a mutual independence among the short-latency responses. Identification remains to be made of a critical cell function which may lie between NGF-receptor binding and the separate branches defining each of the three short-latency events recognized thus far.

243.11 BIOASSAY AND RADIOIMMUNOASSAY OF NGF-LIKE ACTIVITY IN RAT PERIPHERAL NERVE. T. Ebendal* and P.M. Richardson (SPON: G.M. Bray). Dept. of Zoology, Uppsala Univ., Sweden and Div. of Neurosurgery, McGill Univ., Canada.

Three techniques were used to estimate the content of NGF-like activity in normal and injured rat sciatic nerves. Injured nerves were cut at several sites and left in the thigh for 1 - 7 days.

i) Whole sympathetic ganglia from chick embryos were cultured in collagen gels beside peripheral nerve fragments and neurite outgrowth assessed at 48 hours (Brain Res. 246:57, 1982). This method proved highly sensitive although difficult to quantify.

ii) Dissociated sensory neurons from chick embryos were exposed to serial dilutions of nerve homogenates and neurons with neurites were counted at 24 hours (Can. J. Physicl. Pharmacol. 60:707, 1982). The sensitivity of this bioassay was limited by toxic activity of concentrated extracts.

iii) Immunoactivity was measured by a two-site radioimmunoassay with affinity-purified antibodies to ß-NGF from the mouse submandibular gland (Proc. Nat. Acad. Sci. 75:4042, 1978).

In normal nerves, trace amounts of NGF-like activity were detected by the whole ganglionic assay but not by the other two methods. After injury, a transient rise in NGF-like activity maximal at 2 days, was demonstrable by all 3 techniques. By bioassay and radioimmunoassay, the peak concentration of NGF-like activity in injured nerves was approximately 3 fmoles/cm sciatic nerve. The levels of NGF-like activity in normal and injured peripheral nerves appear to be in a physiologically relevant range.

(Supported by Natural Sciences Research Council of Sweden, Medical Research Council of Canada and Multiple Sclerosis Society of Canada.)

243.12 TROPHIC EFFECTS OF SKELETAL MUSCLE EXTRACTS ON NEURITE DEVELOPMENT IN PERIPHERAL GANGLIA. L. Hsu, D. Natyzak* and G.L. Trupin*, Depts. of Anatomy, New Jersey Sch. of Osteopathic Medicine and Rutgers Med. School, Piscataway, New Jersey, 08854.

Previously we have reported on the trophic effects of homogenized skeletal muscle extracts on the differentiation of spinal cord explants (Hsu, L. et al, J. Embry. Exp. Morph. 71, 1982). We now report that skeletal muscle extracts from embryonic chicks also enhances the differentiation of neurites from both dorsal root and sympathetic ganglia.

Within 2 days in vitro, ganglia grown in medium containing muscle extracts developed radial fibers extending over 800µm in length. Individual neurites or small fascicles accompanied by spindle shaped non-neuronal cells extended to form elaborate networks. In contrast, ganglia maintained in control medium with serum proteins only achieved limited neuritic and glial outgrowths. When muscle extracts were used to coat the substratum of either collagen or polyornithine, ganglia maintained on these surfaces in serum-less control medium showed significantly enhanced neuritic development. These studies indicate that the neurite-promoting component of the muscle extract may be mediating its trophic effect by interacting with the underlying substrate. Trophic activity appears to be specific to skeletal muscle since other tissue extracts such as brain were ineffective in enhancing neuritic development when used either as growth medium or as a substrate-conditioning agent. Pretreatment of the plastic dish surface with the cell-attachment factor, fibronectin, also did not promote neuritic outgrowth.

We have begun to characterize the trophic component of skeletal muscle extracts. Treatment with heat (60°C and 90°C), base (0.1N NaOH) or trypsin (0.5mg/ml) reduced the ability of muscle extracts to stimulate neurite outgrowth. Incubation with acid (0.1N HCl) or antisera to nerve growth factor, however, did not affect its trophic properties.

(This work was supported by a grant from the National Osteopathic Foundation)

243.13 INJURY-INDUCED ASTROCYTE MITOGENIC AND TRANFORMATION FACTORS IN RAT BRAIN. M. Nieto-Sampedro, R.P. Saneto, J. de Vellis and C.W. Cotman, Dept. of Psychobiology, Univ. of Cal., Irvine, CA 92717 and Dept. of Anatomy and Psychiatry, School of Medicine, Univ. of Cal., Los Angeles, CA 90024.

We have previously shown that injury to rat brain elicits the appearance in both the tissue surrounding the wound and and in the wound cavity of substances capable of supporting the survival of dissociated neurons in culture (Nieto-Sampedro et al., 1982, Science: 217, 860).

CNS injury is well known to cause the proliferation of fibrous astrocytes in the tissue around the wound. Therefore, we initiated a study to demonstrate the appearance, following injury, of factors capable of stimulating astrocyte division and transformation in vitro. The main findings are as follows.

1. Low levels of mitogenic activity were present in normal rat brain. Following injury, this activity increased over time 3 to 10-fold. Maximum activity was reached 10 days post-lesion in the tissue surrounding the wound and about 15 days post-lesion in the gelfoam filling the wound cavity.
2. Factors capable of inducing the transformation of astrocytes from polygonal-flat morphology to fibrous-like morphology appeared in gelfoam extracts 15 days postlesion, but were not detectable at earlier times.
3. Temperature (50°C) or acid-pH treatments of the brain tissue extracts increased their mitogenic activity. These treatments also unmasked astrocyte transformation activity in the tissue at all times postlesion.
4. Both mitogenic and transformation factors were non-diffusable, and heat and trypsin sensitive, i.e. they had the properties of protein-like substances.

Brain tissue in the area of an injury releases substances that promote neuron survival. We show now that it also releases factors that cause astrocyte proliferation and transformation. The data suggest that the factors are present in the brain in an inhibited form and that injury perhaps releases them from inhibition and/or induces an increase in the synthesis of the active molecules. These factors are presumably responsible for the astrocyte reaction to injury (proliferation and transformation) typically observed in vivo.

Supported by a grant from the California Cancer Research Coordinating Committee to MN-S.

243.14 CHARACTERIZATION OF INJURY-INDUCED NEURONOTROPHIC ACTIVITY IN NEONATE, MATURE AND AGED RAT BRAIN. D.L. Needels* S.R. Whittemore, M. Nieto-Sampedro and C.W. Cotman. Dept. of Psychobiology, Univ. of Calif., Irvine, CA 92717.

Previously, we have demonstrated that substances capable of supporting the survival of peripheral neurons in culture (neuronotrophic factors, NTFs) are present in low levels in the brain of both neonatal and adult rats, and are increased following injury (Nieto-Sampedro et al., Science 217:860).

A comparative study of the induction by injury of NTFs in neonatal, adult and aged rats has revealed the following:

1. Extracts of brain tissue from rats of all ages contained low levels of NTFs that increased following injury.
2. Rat brain NTFs supported the survival of avian ciliary ganglion (CG) neurons, but were 2-4 times more active on cultured neurons from embryonic rat corpus striatum, hippocampus and thalamus.
3. NTF activity, measured using striatal cultures, was about three times higher in normal brain of aged rats than that of young adult or neonatal animals.
4. Induction of NTF activity by injury was slower in aged brain than in young adult or neonate. For both peripheral and central neurons, maximum NTF activity in the brain tissue surrounding a wound was found 7 days post-lesion in neonates, 10 days post-lesion in young adults and 15 days post-lesion in aged animals.
5. These extracts also contain toxic activity for central neurons. Similar levels were found in rats from all ages, and did not appear to change following injury. CG neurons were unaffected by the toxic activity.

NTFs are probably involved in neuronal maintainance and injury repair. Neuronal death, ubiquitous in aged animals, is probably responsible for the increased basal NTF levels in aged brain. As the animal ages, the rate of induction of these factors diminishes, which may be involved in the decreased ability of aged neural tissue to recover from injury.

Supported by NS07032A (SRW) and NS08597 (CWC).

243.15 AN EFFECT OF NERVE GROWTH FACTOR ON THE PARASYMPATHETIC CILIARY GANGLION. F. Collins* (SPON: T. Parks) Dept. of Anatomy Univ. of Utah Sch. of Med., Salt Lake City, UT 84132

We have recently reported that mouse submaxillary nerve growth factor (NGF) at 0.1-10 ng/ml induces a two-fold increase in the rate of neurite elongation within 30 minutes of its addition to cultures of dissociated parasympathetic ciliary ganglion neurons from 8 day chicken embryos. NGF in these conditions has no effect on ciliary ganglion neuronal survival (Collins and Dawson, 1983, Proc.Natl.Acad.Sci.,U.S.A. 80:2091).

The concentration of NGF required to manifest this effect on neurite elongation is within the normal physiological range. We have now iodinated preparations of NGF which elicit this response and found that greater than 99% of the radioactivity detectable on polyacrylamide gels, run in reducing conditions, comigrates as a single band with unlabelled NGF. Greater than 85% of the radioactivity of such preparations can be precipitated using affinity-purified anti-NGF antibody. We are presently using radiolabelled NGF to determine whether ciliary ganglion neurons have binding sites for NGF which might mediate its growth promoting effects.

To answer the question whether the response of ciliary ganglion neurons to NGF is somehow a result of dissociation, we have determined the effects of NGF on undissociated ganglia As previously observed with dissociated cells, whole ciliary ganglia cultured in the presence of a substratum-conditioning factor which promotes neurite outgrowth also show a dramatic response to as little as 1 ng/ml NGF There is a highly significant increase in both the lengths and density of neurites in NGF-treated compared to control ciliary ganglia. As was previously known, NGF has analogous effects on embryonic sympathetic chain and dorsal root ganglia. However, we have observed that in identical conditions, explants of neural retina show no significant change in neuritic density or lengths upon addition of NGF. We are presently examining other neural explants to further determine the specificity of this unexpected effect of NGF.

243.16 CONCENTRATIONS OF α AND γ SUBUNITS OF 7S-NERVE GROWTH FACTOR IN MOUSE SUBMANDIBULAR GLANDS. N.J. Pantazis. Dept of Anatomy, Univ. of Iowa, Iowa City, IA 52242.

Two forms of Nerve Growth Factor (NGF) have been isolated from the mouse submandibular gland, 7S-NGF (Varon, S., Nomura, J. and Shooter, E.M. Biochemistry 6:2202, 1967) and NGF_1 (Young, M., Saide, J.D., Murphy, R.A. and Blanchard, M.H. Biochemistry 17:1490, 1978). Both forms of NGF appear to contain three subunits, called α, γ and β-NGF. The concentration of the β-NGF subunit is known to be approximately 10 times higher in the male gland compared to the female gland and this result was reconfirmed by radioimmunoassay specific for β-NGF. The apparent reason for this difference is that the granular tubule cell which is the site of synthesis of β-NGF in the gland is larger and more numerous in the male. In order to determine whether this same concentration difference was also present for the α and γ subunits, specific α and γ radioimmunoassays (RIAs) were used to determine the gland concentration of both these proteins. The concentration of α was 8-fold greater in the male gland while γ was 9-fold greater Since the male/female concentration ratios for β-NGF, α and γ are so similar, this would suggest that α and γ, like β-NGF, are produced in the granular tubule cells.

However, when β-NGF, α and γ concentrations were compared to each other within the male gland, a surprising result was evident. The ratio of β-NGF:α:γ in the male was 1:3:4 while in the female it was 1:4:5. For comparison, the stoichiometry of 7S-NGF (β-NGF:α:γ) is 1:2:2. This evidence suggests that the concentrations of α and γ are greater than is necessary to bind all of the β-NGF in the gland to form 7S-NGF. There appear to be considerable quantities of α and γ which are not bound to β-NGF and are apparently free in the gland. This is exactly the result seen on gel filtration chromatography (Sephadex G-100) of a male gland homogenate. The column elution position of the 7S-NGF complex with its three subunits was located using RIAs for α, γ and β-NGF. At a lower molecular weight of 25,000 both unattached α and γ were detected but not β-NGF. The advantages if any, of having excess concentrations of α and γ are not clear, but it may possibly be a mechanism of stabilizing the 7S-NGF molecule which is known to dissociate into its subunit components (Pantazis, N.J., Murphy, R.A., Saide, J.D , Blanchard, M H. and Young, M Biochemistry 16:1525, 1977). Finally, this data also indicates that if the synthesis and/or degradation of α, γ and β-NGF are coordinately controlled in the gland, it is done in a way which produces excess concentrations of α and γ

Supported by NIH grants GM 28644 and CA 24241.

243.17 CHOLINERGIC HIPPOCAMPAL NEUROTROPHIC FACTOR:DEVELOPMENTAL CHANGES & RESPONSE TO INJURY. A.M. Heacock, A.R. Schonfeld & R. Katzman Dept. of Neurology, Albert Einstein College of Med.,Bronx, N Y.

While trophic substances required by cultured neuronal cells have been well-studied, their role in CNS development and response to injury has remained speculative. Chick embryo ciliary ganglion cells provide a sensitive bioassay which has revealed cholinergic neurotrophic factors(CNTFs)from many sources. Among these is chick heart conditioned medium(HCM) which we have found to also have effects in vivo on regeneration of the septohippocampal pathway (Schonfeld et al,Brain Res.229:541,1981). If septal neurons normally receive CNTFs from their target tissue, then the hippocampus could be a particularly appropriate source. Recently, (Crutcher & Collins(Sci.217:67,1982) reported that rat hippocampal extract enhanced the rate of neurite extension of chick ciliary ganglion cells. In this report, we describe survival-promoting activity present in rat hippocampus which is found at low levels at birth, reaches a maximum at one month postnatally and increases following injury.

A soluble fraction of adult rat hippocampus was diluted with culture medium, added to culture dishes containing dissociated ciliary ganglion cells and neuronal survival was assessed after 24-48 hr Maximal enhancement of survival was achieved with 30-40 ug hippocampal extract protein. Only sparse neurite outgrowth was observed even at the highest concentration tested(400ug/ml) In contrast, if the culture dishes were pre-coated with neurite-promoting factor from HCM, hippocampal extract supported extensive neurite outgrowth. The CNTF is heat and trypsin sensitive and is also present in cortex and cerebellum.

A possible correlation with developmental events was examined by determining trophic content of the hippocampus postnatally Activity is expressed in trophic units(TU) which is the amount supporting one-half maximal survival. During the first two weeks of life, there was no change in trophic activity from the level of 25 TU/mg protein at birth. By day 33, the adult level of 65 TU/mg protein had been reached. Such a time course, while not consistent with a neurotropic role for hippocampal CNTF in early development, may suggest a glial origin. Preliminary data indicate that there is no change in hippocampal CNTF with aging(1 and 2 yr)

The response of CNTF to injury was then determined. A small suction lesion of the anterior hippocampus produced a two-fold increase in trophic content in the tissue surrounding the wound and led to the accumulation of CNTF in the wound cavity fluid. A physiological relevance for this hippocampal CNTF measured in vitro is supported by the effects in vivo of a prior lesion of the hippocampus on CNS cholinergic regeneration (see Schonfeld et al, following abstract).

Supported by a Potamkin-Lerner Fellowship.

243.18 CHOLINERGIC HIPPOCAMPAL NEUROTROPHIC FACTOR: EFFECTS ON CNS REGENERATION IN VIVO. A.R. Schonfeld, A. Heacock and R. Katzman. Dept. of Neurol., Albert Einstein Col. of Med., Bronx, NY.

The hippocampus of adult rats contains at least one neurotrophic factor (NTF) capable of accelerating the growth of cholinergic parasympathetic neurons in vitro (Crutcher and Collins, Sci. 217: 67, 1982). Injury to the brain stimulates the accumulation of NTF(s) released from neural tissue and the trophic activity of these factors for cholinergic neurons in vitro increases within days following trauma (Nieto-Sampedro et. al., Sci. 217:860, 1982), In order to elucidate a role for NTFs in vivo, two experiments were undertaken to determine if (1) intracerebral administration of exogenous hippocampal NTF or (2) post-traumatic release of endogenous hippocampal NTF enhance the regeneration of injured, mature cholinergic axons in rat brain.

In the bioassay used for these studies, iris tissue implanted into the anterior hippocampus serve as targets for newly regenerating cholinergic septohippocampal fibers following transection (Svendgaard et. al., Brain Res. 102:1, 1976; Emson et. al., Brain Res. 135:87, 1977). Levels of choline acetyltransferase (CAT) within implants are used as an index of cholinergic regeneration (Emson et. al , 1977). In Exp. I, a cannula was stereotaxically placed in the medial septum at the same time that an iris was inserted unilaterally in the anterodorsal hippocampus of adult female Sprague-Dawley rats. Animals were administered 1 µl of either adult rat hippocampal extract (n=7) or control vehicle (n=8) daily. Eight days later, rats were killed by decapitation, the brains rapidly removed, irides dissected free from surrounding parenchyma, weighed, frozen and assayed for CAT according to Fonnum (J. Neurochem., 24:407, 1975). The results indicated no difference between treatments (CAT nmoles/mg/hr: $\bar{x}$ control = 4.84 $\pm$ 0.85; $\bar{x}$ hippocampal extract = 4.78 $\pm$ 0.74).

In Exp. II, 6 rats received a lesion produced by insertion of a fine glass rod in the anterodorsal hippocampus 1 week prior to iris implantation in the same location; 5 control animals were not lesioned prior to iris implantation. Eight days later, irides were removed and assayed for CAT. The results revealed a significant difference between groups for both CAT (CAT nmoles/mg/hr: $\bar{x}$ control = 2.01 $\pm$ 0.42; $\bar{x}$ lesion = 9.25 $\pm$ 1.16; t=5.43, df=9, $p<0.001$, t test) and protein (protein mg/10λ: $\bar{x}$ control = 0.0102 $\pm$ 0.0006; $\bar{x}$ lesion = 0.0163 $\pm$ 0.009; t=5.73, df=9, $p<0.001$, t test)

These observations and those of the preceding abstract (Heacock et al.) suggest that, under selective circumstances, NTFs contained in neural tissue are active in vivo. Differences in the results of Exps I and II may reflect injury-induced enhancement of trophic potency or differential sensitivity of cell soma and transected axonal endings to NTFs. (Supported in part by the Wood Kalb Foundation)

243.19 METHYLATION INHIBITORS BLOCK RESPONSES OF PC12 PHEOCHROMOCYTOMA CELLS TO NERVE GROWTH FACTOR (NGF), BUT NOT TO EPIDERMAL GROWTH FACTOR (EGF). P. J. Seeley*, A. Rukenstein*, J.L. Connolly*[a] and L. A. Greene* (SPON: A. Chalazonitis). Dept. of Pharmacology, NYU Sch. Med., New York, NY 10016 and [a]Dept. of Pathology, Harvard Med. Sch., Boston, MA 02115.

In an effort to uncover transductional steps in the mechanism of action of NGF, rat PC12 cells were assayed for responses to NGF and EGF in the presence and absence of varying concentrations of cellular transmethylation inhibitors. The agents used included 5'-deoxy-5'-methylthioadenosine (MTA), 5'-deoxy-5'-isobutylthioadenosine (SIBA), and 3-deazaadenosine (DAA) + homocysteinethiolactone (HCTL). HCTL potentiates the anti-methyl-transferase activity of DAA. Each inhibitor blocked rapid (i.e. occurring within 24 h), NGF-dependent neurite regeneration. HCTL alone was without effect but significantly potentiated the inhibitory action of DAA. Reversibility of the inhibitors was demonstrated by the reappearance of neurites following washout. Initiation of neurite outgrowth in response to NGF, which slowly occurs over several days, was also reversibly suppressed by MTA and SIBA. Moreover, reversible blockade occurred in the priming response, a transcription-dependent component of the NGF mechanism responsible for neurite outgrowth. Additional responses of the cells were examined that are promoted by both NGF and EGF. These included: 1) Rapid reorganization of surface architecture (i.e. loss of microvilli, transient ruffling) that is detectable within 30 sec of factor exposure. 2) Enhancement, within 15 min, of the phosphorylation of certain cell proteins, the most prominent of which is tyrosine hydroxylase. 3) Transcription-dependent induction of ornithine decarboxylase activity which is maximal by 4-6 hr of treatment. The inhibitors alone did not mimic these responses. In each case, the actions of NGF were suppressed by the inhibitors; in contrast, under the same conditions, each of the actions of EGF was either unaffected or significantly enhanced. The latter findings appear to discount the possibility that inhibition of responses to NGF was due to non-specific effects on cell function or to toxicity. HCTL alone was ineffective, but again potentiated the suppression of NGF responses by DAA. This is consistent with the possibility that the inhibition of NGF actions was due to blockade of transmethylation. One interpretation of these findings is that transmethylation (either ongoing or factor-stimulated) plays a necessary role in the action of NGF, but not of EGF. The observation that a variety of different responses to NGF were blocked, including those that are rapidly induced, suggests interference with one of the first steps in the mechanistic pathway of NGF. Supported by grant from the NIH (NS 16036 and AM 26920).

243.20 NERVE GROWTH FACTOR SYNTHESIZED BY MOUSE S-180 CELLS IN CULTURE. I. Kim* and N.J. Pantazis (SPON: J. Jew). Dept. of Anatomy, Univ. of Iowa, Iowa City, IA 52242.

Nerve Growth Factor (NGF) is synthesized by several different types of cells in culture. Do cells in culture produce NGF molecules similar to those seen in the mouse submandibular gland, the most potent source of NGF? Two forms of NGF have been isolated from the submandibular gland, 7S-NGF (Varon, S., Nomura, J. and Shooter, E.M., Biochemistry, 6: 2202, 1967) and NGF_1 (Young, M., Saide, J.D., Murphy, R.A. and Blanchard, M.H., Biochemistry, 17:1490, 1978). Both molecules apparently contain three subunits designated α, γ and β-NGF. Studies using specific radioimmunoassays (RIAs) for α, γ and β-NGF determined that neither α nor γ was present in medium conditioned by fibroblasts whereas β-NGF was readily detected (Pantazis, N.J., submitted to Biochemistry). Since two of the three subunits contained in 7S-NGF and NGF_1 were not present, this indicates that neither of these forms of NGF is produced by mouse fibroblasts.

A recent paper (Barklis, E. and Perez-Polo, J.R., J. Neurosci. Res., 6:21, 1981) suggested that mouse S-180 cells in culture produce a 7S-NGF molecule. Although the S-180 cell line is now called a sarcoma, it was originally characterized as an axillary carcinoma (Steward, H.L., Snell, K.C., Dunham, L.J. and Schlyen, S.M., Transplantable and Transmissible Tumors of Animals, p. 243, Armed Forces Inst. Pathol., Sec. 12, F40, Washington, D.C., 1956). S-180 cells were investigated to establish whether they are different from mouse fibroblasts and produce 7S-NGF. Radioimmunoassays of concentrated samples of S-180 cell conditioned medium detected little or no α or γ protein while β-NGF was present in significant quantitites (700-800 ng/ml). NGF bioassays indicated that the S-180 β-NGF was biologically active. The β-NGF produced by S-180 cells was examined on denaturing (5M guanidine hydrochloride) gel filtration columns and its molecular weight was identical to that of submandibular gland β-NGF. The S-180 β-NGF was further characterized on isoelectric focusing gels. The isoelectric point of the S-180 β-NGF was indistinguishable from that of submandibular gland β-NGF.

In conclusion, S-180 cells synthesize a β-NGF protein similar to that found in the mouse submandibular gland. However, radioimmunoassays were unable to detect α and γ. As in the case with the mouse fibroblasts, these results would suggest that neither 7S-NGF nor NGF_1 is produced by S-180 cells in culture.

Supported by NIH grant GM 28644 to N.J.P.

243.21 MOTOR NEURON SURVIVAL AND NEURITE GROWTH: PROMOTION BY FACTORS FROM DIFFERENT CELL TYPES IN EMBRYONIC MUSCLE. V. Nurcombe*, S. Tout* and M.R. Bennett. Neurobiology Research Centre, University of Sydney, N.S.W. 2006, Australia.

Embryonic chick skeletal muscle releases both a motor neuron survival factor and a neurite expression factor in vitro (Nurcombe and Bennett, J. Comp. Neurol., in press 1983). The present experiments were designed to identify the cell type within skeletal muscle which is primarily responsible for these neurotrophic effects; the cell types studied were myoblasts, myotubes, fibroblasts, endothelial cells, undifferentiated mesenchyme and Schwann cells. Each cell type was prepared from skeletal muscle of the appropriate embryonic age (ranging from 4-10d of gestation) and grown to confluence in monolayer culture. Serum-free conditioned media (CM) obtained from these cultures were assayed for survival- and neurite-promoting properties against dissociated cultures of HRP-labelled motor neurons (MN; Bennett et al., Brain Res. 190; 1980). The MN were seeded onto polyornithine plates in CM diluted with standard medium containing 5% fetal calf serum (FCS). The proportion (%) of MN which survived and the proportion which expressed neurites were counted after 72h. Myotube CM was found to promote neuronal survival to levels over twice as high as the next most effective cell types, the muscle fibroblast and the myoblast. All CM were found to trigger neurite outgrowth; again, the greatest levels were triggered by the myotube CM.

Further experiments were performed to assess the effects of different cell types in embryonic muscle on neurite expression from organotypic explants of the lateral motor column. These explants were dissected from 8-9d embryos and tested against each of the CM; the CM was diluted with 0.5% FCS and the explants incubated for 5d. Myotube CM again enhanced neurite outgrowth to levels at least twice that of the next most effective cell type, the fibroblast.

These results support the hypothesis that in vivo myotubes release a neurite promotion factor which directs the advancing growth cone towards it (Bennett et al., J. Comp. Neurol., 215, 1983); they also support the possibility that myotubes provide a survival factor for the MN (Eagleson & Bennett, Neurosci. Lett., in press 1983).

243.22 SCHWANN CELL PROLIFERATION AND PROTEOLYSIS: REGULATION BY THE PLASMIN-GENERATING SYSTEM. N. Kalderon. The Rockefeller Univ., New York, 10021.

Schwann cells proliferate during development of the nervous system and in the adult nervous system upon regeneration as a result of nerve injury. This cell proliferation entails remodeling events in the tissue as the new neuronal-Schwann cell interactions are established. The role of the serum proteolytic system, plasminogen/plasminogen activator, in the regulation of nervous tissue remodeling processes in the proliferation of Schwann cells is being studied.

It is reported in this study that purified dividing Schwann cells in culture produce extracellular plasminogen activator, as measured by the biochemical fibrinolytic assay (details in Kalderon, 1979, PNAS, 76:5992). Purified Schwann cells in culture divide at a low rate. Recently a growth factor (GGF) from bovine pituitary was isolated which enhances Schwann cell proliferation (Brockes et al., 1980, J.B.C. 255:8274). However, fetal calf serum (FCS) is an essential component in the medium for its action (Raff, et al., 1978, Cell, 15:813). For our studies, purified Schwann cell cultures are obtained from the sciatic nerve of newborn mice (Brockes et al., 1979, Brain Res. 165:105), and are maintained in Dulbecco's minimal essential medium (DMEM) supplemented with 10% FCS and partially purified GGF from the bovine pituitary (Brockes et al., 1980). When the above medium is replaced with a chemically defined medium with or without the GGF, the Schwann cells proliferate at a minimal rate. The chemically defined medium is composed of DMEM supplemented with: 5 μg/ml bovine insulin, 5 μg/ml human transferrin, 20 nM progesterone, 100 μM putrescine dihydrochloride, 30 nM sodium selenite, 100 μg/ml bovine serum albumin. However, addition of plasminogen (0.05-1 μg/ml) with the GGF to the chemically defined medium was sufficient to stimulate Schwann cell division (3-4 times) to a rate similar to the one in the DMEM + 10% FCS (as measured by [^{3}H]thymidine uptake). The effect of plasminogen is partially blocked by the protease inhibitor trasylol; however, plasminogen without the GGF does not have any mitogenic effect.

It is concluded that the plasmin proteolytic activity (generated by the activation of plasminogen) is one of the serum components, required by the GGF to promote Schwann cell proliferation. However, it is not clear yet whether other serum components, e.g., protease inhibitors are also needed for the regulation of Schwann cell proliferation.

Supported by grants from NIH NS17169 and Muscular Dystrophy Association.

243.23 ASSOCIATION OF ^{125}I-NGF WITH PC12 PHEOCHROMOCYTOMA CELLS: EVIDENCE FOR INTERNALIZATION VIA HIGH-AFFINITY RECEPTORS ONLY AND FOR LONG-TERM REGULATION BY NGF OF BOTH HIGH- AND LOW-AFFINITY RECEPTORS. P. Bernd and L. A. Greene*. Dept. of Anatomy, Mt. Sinai Sch. of Med., New York, N.Y. 10029 and Dept. of Pharmacology, N.Y.U. Sch. of Med., New York, N.Y. 10016.

Association of ^{125}I-NGF with monolayer cultures of PC12 pheochromocytoma cells was studied under the same growth conditions (RPMI Medium + 15% serum) in which the cells exhibit numerous NGF responses (i.e. cessation of mitosis, neurite outgrowth, etc). Surface bound and internalized factor were distinguished from one another by differential release of the former at low pH, high salt (0.2M acetic acid, 0.5M NaCl). The validity of this approach was verified by EM radioautography and by the absence of non-acid-releasable binding when internalization was blocked at 4°C. ^{125}Binding to the surface was rapid; at 0.2 nM (5 ng/ml) of ^{125}I-NGF, binding to naive cells (NGF-untreated) reached a plateau within 2-5 min. Internalization, in contrast, as revealed by both EM radioautography and scintillation counting, did not start until approximately 2 min after exposure to NGF. Thereafter, internalization proceeded linearly for at least 1/2-1 hr. By the latter time approximately 75% of total bound NGF was within rather than on the surface of the cells. Binding vs. NGF concentration experiments and Scatchard analysis indicated 2 distinct classes of surface binding sites. For both naive cells and cells treated with NGF for at least a week (primed cells), about 7% of the sites had an apparent K_D of 0.3 nM; the remaining sites half-saturated at about 4 nM NGF. The number of each type of site was 3-fold higher/mg protein in the primed cells. For both naive and primed cultures, internalization appeared to be mediated by a single class of uptake sites which half-saturated at ~0.3 nM. Pulse-chase experiments indicated that NGF bound to the high affinity receptors is internalized with a half-time of 3-4 min. The maximal rate of uptake by primed cells (~200 fmol/hr/mg protein) was about twice that for naive cells. Light and EM radioautography indicated that the density of binding was substantially higher in primed cultures and that this increase took place over a time course of days to weeks. These findings suggest that NGF brings about long-term increases in its own receptors and are consistent with a model in which NGF binds to both high- and low-affinity surface receptors, but is internalized only via the high affinity sites. Supported by grants from the NIH (NS 16036), March of Dimes, and Pharmaceutical Manufacturers Association Foundation.

DEVELOPMENT AND PLASTICITY: TROPHIC INTERACTIONS II

244.1 MITOGENIC EFFECTS OF RETINAL EXTRACTS ON RADIAL GLIAL CELLS WITHIN ISOLATED TECTAL TISSUE IN VITRO. Deborah B. Henken and Myong G. Yoon. Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4J1.

Approximately five weeks following unilateral optic nerve crush (ONC) in adult goldfish, enhanced mitosis of radial glial cells was found in the contralateral (conditioned) tectum in comparison with the ipsilateral (normal) tectum (Stevenson, J.A., Br. Res., 153:345, 1978). At about this time, regenerating optic fibers begin to reinnervate their target tectal tissue. The present work aims to determine whether these two events are simply a temporal coincidence or whether the optic fibers exert mitogenic effects on the tectal radial glial cells. To test the latter possibility, we applied either extracts of regenerating retina or extracts of intact retina to normal tectal tissue. We then examined their differential effects on mitosis of radial glial cells.

Previous work in our laboratory has shown that isolated tectal tissue can incorporate exogenous tritiated thymidine into DNA and that this tissue remains viable for up to 125 hours in a biochemically defined culture. To assess whether a mitogenic factor is produced by the retina during tectal reinnervation, extracts were prepared from regenerating retina 35 days following ONC (experimental) and from intact retina (control). Particulate and soluble portions of the extracts were separated in both cases and introduced individually to culture dishes containing normal optic tectum. After 15 to 18 hours of incubation, tritiated thymidine was added to the culture in order to label proliferating cells. The tissue was processed for autoradiography.

Preliminary observations suggest that radial glial mitosis within a normal tectal slice is enhanced by extracts from regenerating retina as compared to normal retinal extracts.

(Supported by grants from NSERC and MRC of Canada)

244.2 RETINAL NEURITE OUTGROWTH STIMULATED BY EXTRACTS FROM ANOPHTHALMIC EMBRYOS. N.G. Carri and T. Ebendal*. Department of Zoology, Uppsala University, Box 561, S-751 22, Uppsala, Sweden.

An essential quality of the visual system is the precise arrangements of its axonal connections. Several mechanisms may be involved in creating these trajectories. An aspect currently studied is wheter trophic interactions(s) and trophic factor(s) may be attributed a role in the development of the visual system. In particular molecular factors regulating the growth of optic axons in culture interest us.

We have earlier shown a trophic effect by the normal optic lobe on the fibre growth from retinal neurons using a short-term bioassay (Soc. Neurosci. Abstr., Vol. 7, p 547, 1981). We now tested the effect of optic lobe missing its supply of retinal axons from anophthalmic chick embryos in the same bioassay. Chick retinal explants (white Leghorn) from 6-day-old embryos were cultured on collagen gels. Tissue extracts (1 gram of tissue in 5 ml of Eagle's Basal Medium, BME,) were dialysed and added to each dish (0.8 ml). They were incubated four days and maximum neurite lenghts were measured in an inverted microscope at 125 x magnification. The extracts (complete optic lobe from normal (OLN) and anophthalmic (OLA) chick embryos 18-day-old) were tested at a series of concentrations equivalent to 100-0.16 mg tissue per ml. Both extracts increased retinal response but the stimulation was about 40% better with OLA. The length of fibers as a function of the concentration of optic lobe extracts (OLN and OLA) were found to fit a sigmoid curve. The stimulative effect of OLA was also evident under serum free conditions.

The results show that extractable macromolecules from target areas stimulate the growth of retinal neurons and suggest that this effect may be greater in a target deficient in its innervation. The data suggest that the trophic interaction(s) during embryonic neural development could be regulated by feedback interactions.

N.G. Carri is a Fellow of CONICET, Argentina.

244.3 RETROGRADE IMPULSE ACTIVITY IN REGENERATED NERVE FIBERS IN NEUROMA STUDIED IN VITRO. J. D. Kocsis and R. J. Preston*. Dept. of Neurology, Stanford University Medical School, and Veterans Administration Medical Center, Palo Alto, CA 94304.

Regenerating nerve fibers that are prevented from reaching their peripheral targets by nerve ligation can give rise to neuroma formation. It has been observed from extracellular unit studies in vivo that activation of a nerve trunk leading into a neuroma can lead to impulses that propagate centripetally. One suggested mechanism for this phenomenon is an "ephaptic" interaction resulting from close apposition of regenerating axon sprouts within the neuroma (Seltzer and Devor, Neurology 29, 1979). Another possibility is retrograde axonal sprouting of the regenerating fibers. In the present study we characterized retrograde impulse activity in an in vitro neuroma preparation, and then used a horseradish peroxidase (HRP) labelling technique to determine the extent of retrograde sprouting. Neuroma formation was induced by ligation of the sciatic nerves of Wistar rats 2 to 16 weeks before the nerves were removed, desheathed, and placed in an in vitro nerve recording chamber. The nerve trunk (1.0 to 1.5 cm) leading into the neuroma was split longitudinally. Stimulation or whole nerve recordings could be obtained from either branch. Stimulation of one branch leading into the neuroma gave rise to impulse activity not only within that branch, but also in a neighboring nonactivated branch. The two branches were connected only through the neuroma head. The response in the activated branch was a discrete short-latency negativity followed by temporally dispersed low amplitude negative components. Only low amplitude late activity was recorded from the nonstimulated branch, and it was abolished by an acute crush at the neuroma neck. From nerves where the interaction was demonstrated, one branch of two leading into the neuroma was dipped in HRP (3% in Kreb's solution) for 1 to 2 hours. The nerves were kept for 24 to 40 hrs at 5° C in Kreb's solution and then fixed in glutaraldehyde, transferred to PO_4 buffer, and processed for HRP histochemistry. HRP stained axons from the stained branch were seen entering and ramifying within the neuroma. When axons reached the distal end of the neuroma, they looped and coursed in a retrograde direction, but in most preparations did not enter the non-dipped branch although impulses were shown to propagate into this branch via the neuroma. However, in some preparations a few HRP stained axons were observed coursing in the non-dipped branch. These results demonstrate that while retrograde impulse activity can be recorded in the absence of morphological evidence for retrograde regeneration in neuroma, one cannot discount the possibility of some retrograde regeneration as contributing to the observed retrograde impulse activity. Supported by the National Multiple Sclerosis Society and the Veterans Administration.

244.4 ULTRASTRUCTURAL PROPERTIES OF CHEMICALLY FUSED PC12 CELLS: EFFECTS OF NERVE GROWTH FACTOR. K. Morrison-Graham and P.H. O'Lague. Dept. of Biol. and Jerry Lewis Neuromuscular Research Center, UCLA, Los Angeles, CA 90024.

Cells of the neuron-like clone PC12 fuse upon exposure to polyethylene glycol (PEG) to produce large multinucleate cells. These cells exhibit many properites of their unfused counterparts including the ability to extend neurite-like processes in response to Nerve Growth Factor (NGF; O'Lague and Huttner, PNAS, 77:1701, 1980). We have compared the ultrastructural properties of fused and unfused cells. Individual cells, previously identified as fused in the phase-contrast microscope, were shown to be fused at the ultrastructural level. All nuclei of fused cells were encased within one plasmalemma and their cytoplasmic constituents were qualitatively similar to those of unfused cells. In several cases evidence for fusion of nuclei was obtained, e.g., unusually large nuclei with more than 2 nucleoli. In fused cells mitochrondria and Golgi appeared normal, and chromaffin-like granules, a striking feature of unfused cells, were common. The chromaffin granules were similar to those of unfused cells in both size (40-170nm in diameter) and appearance.

The processes and growth cones exhibited by the fused cells in the presence of NGF were similar to those of the unfused cells (see also, Luckenbill-Edds et al., J. Neurocytol., 8:493, 1979) and to those of rat sympathetic neurons in vitro. Random cross-sections through fused and unfused cell processes revealed a wide range of process diameters (0.3-3.9μm). The cytoplasmic organization of these processes was similar although larger processes contained proportionally more microtubules and other organelles. The chromaffin granule diameters did not differ from those found in the cell bodies of non-NGF treated cells.

In cell bodies a qualitative increase in the number of rough endoplasmic reticulum (RER) cisternae was observed following a one week exposure to NGF (fused cells 10/14; unfused cells 13/25). In approximately 50% of the cells the cisternae were organized into roughly parallel stacks. These stacks were composed of 3-16 cisternae separated by ~0.1-0.2μm. Numerous free ribosomes were located in the cytoplasmic matrix between the cisternae. These orderly arrays resembled the Nissl substance of neurons. The time course was examined in unfused cells; 15% of the cells had an increase in the RER following a one day exposure to NGF while 86% of the cells showed an increase by 14 days. At this time 84% of the cells had RER organized into stacks.

In summary, these ultrastructural results provide further evidence for the usefulness of the fused PC12 cells as a neuronal model in studies of NGF action. (Supported by NIH grant NS-12901, MDA Center Grant, and a Giannini Foundation Fellowship to KM-G)

244.5 A COLLAGEN INDUCING FACTOR FROM EMBRYONIC BRAIN INDUCES PROLYL HYDROXYLASE ACTIVITY IN CULTURED MUSCLE CELLS. Z. Vogel, D. Duksin* and C. Kalcheim*(SPON: V. Teichberg). Depts. of Neurobiology and Biophysics, The Weizmann Institute of Science, Rehovot 76100, Israel.

Extracts from embryonic rat brain contain a factor which stimulates the production of collagen by cultured rat muscle cells. The newly formed collagen has a role in the aggregation of acetylcholine receptors on the surface of the cultured myotubes (Kalcheim et al., PNAS 79: 3077, 1982; J. Biol. Chem 257: 12722, 1982). Here we report that the collagen-inducing factor is found in extracts of embryonic rat and bovine brains as well as in embryonic spinal cord extracts. Some activity was also detected in embryonic kidney, but no activity was found in extracts of adult brain and placenta or in embryonic rat muscle, lung, liver and skin.

The collagen-inducing factor from embryonic brain was partially purified by membrane and gel filtration. The activity was eluted from Biogel P-10 or Sephadex G-25 columns (in 10mM HEPES, 150 mM NaCl, 5mM KCl, pH 7.4) as a single peak with an apparent molecular weight of around 700 daltons. The partially purified factor induced the production of collagenous proteins of types I, III, IV and V which were deposited on the surface of the treated myotubes in a distinct pericellular pattern.

The increased secretion of collagenous proteins is accompanied by a fifteen-fold increase in the hydroxyproline-to-proline ratio in secreted proteins (29% compared to 1.9% in controls) and a five-fold increase in the above ratio in cellular proteins (8.9% compared to 1.7% in controls). Moreover, a five- to fifty-fold increase in the activity of the enzyme prolyl hydroxylase was found in extracts derived from factor-treated cultures compared to controls. No stimulatory effect on prolyl hydroxylase activity was detected when the factor was exogenously added to cell homogenates. The procollagen mRNA level was not affected by treating the cultures with the factor. In addition, the level of activity of the muscle enzyme creatine phosphokinase was also unaffected.

On the basis of the above data we suggest that the effect of the neuronal factor which stimulates the production of collagen is achieved by inducing the synthesis or the activation of the enzyme prolyl hydroxylase in the cultured cells. (Supported by a grant from the Muscular Dystrophy Association).

244.6 INDUCTION OF SPINAL CORD ANDROGEN RECEPTORS BY MUSCLE FACTORS. C. L. Weill. Departments of Neurology and Anatomy, Louisiana State University Medical Center, New Orleans, LA 70112.

Motoneurons of the lateral motor column in the spinal cord of chick embryos have been shown to accumulate ^{3}H-dihydrotestosterone implicating the presence of androgen receptors (Exp. Brain Res. 44:243, 1981). Which cellular functions are regulated by these receptors is unknown. Studies were carried out to determine if these receptors are under trophic regulation by factors from skeletal muscle. Spinal cord cultures were prepared by plating dissociated 6-day old chick embryo spinal cord cells at a density of 3 x 10^6 cells/ml in collagen coated multiwell dishes. The number of nonneuronal cells in these cultures was minimized by plating in the presence of 10^{-5} M cytosine arabinoside for the first 48 hr. Muscle-conditioned medium (M-CM) was obtained from mature skeletal muscle cultures prepared from 12-day old chick embryo leg muscle myoblasts plated at a density of 4 x 10^6 cells/ml. Binding of the androgen, ^{3}H-methyltrienolone (^{3}H-R1881), was determined on intact cultures at 37° C using a monolayer assay procedure.

Specific ^{3}H-R1881 binding reached equilibrium in 2 hr and increased linearly with cell number over the range of 0.5 - 2.0 x 10^6 cells per determination. Specific binding was saturable and was inhibited by unlabeled R1881, testosterone and dihydrotestosterone, but not by dexamethasone and beta-estradiol. Specific binding to cytosols prepared from neuronal cultures was abolished by heating the cytosol at 100° C for 10 min or treating the cytosol with trypsin.

Spinal cord cultures maintained in MEM containing 10% horse serum and 5% chick embryo extract contain measurable levels of androgen receptor in the range of 117 +/- 10 fmole/mg protein (m +/- S.E., n=3). Dose response curves for M-CM indicated an optimum concentration of 40%. When treated with 40% M-CM for 4-6 days spinal cord cultures displayed a 2.00 +/- 0.36 fold (M +/- S.E., n=3) increase in specific ^{3}H-R1881 binding.

These data demonstrate that factors produced by skeletal muscle maintained in culture are able to cause an increase in the number of available androgen receptors in cultured spinal cord cells. This taken together with the accepted mechanism of action of steroid receptors suggests that target muscle may elicit its neurotrophic effects through steroid hormone mediated selective gene expression. (Supported by grants from the Amyotrophic Lateral Sclerosis Society of America and The Schlieder Foundation)

244.7 NUCLEAR ORGANIZATION AND CELL DEATH IN THE CHICK OCULMOTOR NUCLEUS FOLLOWING TARGET ALTERATION. D. B. Wayne* and M.B. Heaton. Dept. of Neuroscience, University of Florida College of Medicine, Gainesville, FL, 32610.

The oculomotor nucleus innervates four of the extraocular muscles. These muscles are derived entirely from mesoderm while the ciliary ganglion and connective tissue enveloping the muscle are neural crest derived. Within the nucleus, there is a segregation of motoneuron populations which innervate the different muscles (Heaton, M.B. and D.B. Wayne, J. Comp. Neurol., 217(3): in press, 1983). During normal development this segregation of motoneurons is produced by the migration of a subpopulation of cells and the separation into subnuclei. This is followed by a period of normally occurring cell death. The morphological arrangement of motoneurons in the absence of their normal target was examined during the period of developmental organization and following the period of cell death.

Following early removal of the paraxial mesoderm and neural crest the early developmental organization progressed as normal. In the twenty-one cases examined between 5-10 days of development both cell migration within the nucleus and the separation into distinct subnuclei occured. The configuration of the experimental nucleus mirrored that of the control nucleus despite a virtual elimination of the target muscle in some cases. Also, the nuclear organization did not seem to rest on the presence of neural crest derived tissue as indicated by the depletion of the ciliary ganglion. At the end of the period of normal cell death, day 16, virtually all the motoneurons deprived of their target have disappeared. Only a small population of cells remain along the midline on the experimental side. These are presumably the neurons which migrate across the midline during early development and provide innervation to the contralateral superior rectus muscle. It is the complete absence of cell groups and the location of the remaining cells which is of interest here rather than a quantification of the amount of cell death.

In a few cases the experimental manipulation caused the nerve to deviate from its normal course. In these cases the nerve crossed to innervate the muscle on the intact side. This yielded an effective reduction in the quantity of muscle available and altered any lateralized cues. As previously, the organizing events in the nucleus, migration and subnuclear separation, occurred as normal. The effect of this condition on the rate of cell death is currently under investigation.

244.8 GROWTH OF C-1300 NEUROBLASTOMA IN TISSUE CULTURE IS AUGMENTED IN THE PRESENCE OF SYMPATHETIC GANGLION EXPLANTS. E. Chelmicka-Schorr*, R.C. Yu, M.G. Sportiello* and B.G.W. Arnason. Brain Research Institute and Dept. of Neurology, Univ. of Chicago, Chicago, IL 60637.

We have shown previously that growth of C-1300 neuroblastoma (C-1300 NB) is significantly suppressed in mice sympathectomized or axotomized by pretreatment with 6-hydroxydopamine. When newborn mice were pretreated with nerve growth factor (NGF) to induce neuronal hypertrophy, neuronal maturation, and peripheral hyperinnervation of the sympathetic nervous system (SNS), the growth of neuroblastoma was augmented. These experiments suggest a modulatory role of the SNS on C-1300 NB growth (Chelmicka-Szorc, E., Canc Res ,36:382, 1976; Chelmicka-Schorr, E., Canc Res 38:1374, 1978). To explore this phenomenon, we cocultured sympathetic ganglia (SG) and C-1300 NB explants or S-20 NB cells. Superior cervical ganglia (SG) were taken from 1-day old rats and cultured in collagen-coated petri dishes. The culture medium was DMEM enriched with 10% FBS and NGF (84 ng/ml). Cultures were maintained at 37°C in humidified 5% CO_2 balanced with air. After 72 hours, 3-4 explants of C-1300 NB were added to petri dishes containing 2-4 well established SG that had extended multiple processes. Control cultures had either SG or C-1300 NB, both in the medium described above. The presence of SG in NB containing dishes significantly augmented growth of C-1300 NB cells as compared to C-1300 NB grown alone. The difference was easily noticeable 48 hours after coculturing and was striking 12 days later when proliferating C-1300 NB cells covered almost the entire petri dish. There was only sparse growth of cells from C-1300 NB explants grown without SG. Density of the C-1300 NB cells was significantly greater in the proximity to SG. A similar experiment was done with dispersed S-20 NB cells, a clonal line derived from C-1300 NB. An increased density of S-20 cells was again seen in proximity to SG. The growth of the SG themselves was not changed in the presence of C1300 NB or S-20 NB in the culture. We postulate that a trophic factor which increases the proliferation of NB cells is secreted by sympathetic ganglia. This finding would explain why growth of C-1300 NB in vivo is suppressed in mice with ablated SNS.

This work is supported by a Grant PHS 1 RO1 NS 18413-01 (Dr. Chelmicka-Schorr) and by a grant from The University of Chicago Brain Research Foundation (Dr. Riley Yu).

244.9 EVIDENCE FOR THE POSTINSERTIONAL STABILIZATION OF ACh RECEPTORS AT THE NEUROMUSCULAR JUNCTION. E. F. Stanley, and D. B. Drachman. Department of Neurology, Johns Hopkins Sch. of Med., Baltimore, MD 21205.

ACh receptors at innervated neuromuscular junctions are known to be very stable, with an average half-life of about 10 days in the mouse. However, denervated junctions have ACh receptors that are rapidly turned over, with a half-life of about 1 day (Levitt and Salpeter, Nature 291:239, 1981) similar to those that appear at extra-junctional regions of muscle fibers after denervation. We have recently reported that normal neuromuscular junctions have two populations of receptors: 1) a large sub-population (80%) of stable receptors, and 2) a small but significant sub-population (20%) that are rapidly turned over (RTOs)(Soc Neurosci Abstr 8:185, 1982). We have postulated that the RTOs are the precursors of the stable receptors, and that the stabilization process may depend on an influence from the motor nerve.

In the present study, we have tested this hypothesis in the mouse flexor digitorum brevis muscle by: 1) labeling junctional ACh receptors with ^{125}I-α-BuTx, 2) denervating by cutting the sciatic nerve, and 3) following the fate of the RTOs through a 6 day period when they were either degraded or converted to stable ACh receptors. Our hypothesis predicts that denervation would prevent the conversion of RTOs to stable receptors, and would result in a reduced number of stable AChRs in the denervated muscles as compared with the innervated muscles.

Details of the experimental procedures will be presented. The results show that there were more ACh receptors remaining in the innervated muscles than in the denervated muscles at the end of the 6 day period ($p < .001$). This was attributable to failure of conversion of the RTOs to stable receptors following denervation. Control experiments showed that this could not be accounted for by acceleration of degradation of the stable receptors.

Our results are consistent with the hypothesis that the RTOs are precursors for the stable receptors, and that an intact motor nerve supply is necessary for their conversion to stable receptors.

244.10 EXPERIMENTALLY LENGTHENED NERVES SHOW THAT NEUROTROPHIC MAINTENANCE DEPENDS UPON AXON LENGTH B. Oakley, E. Keppel and S. E. Hughes. Dept. of Zoology, Neuroscience Lab. Bldg. Univ. of Michigan, Ann Arbor, MI 48109.

The neurotrophic dependence of end organs can be demonstrated by nerve transection. Characteristically, longer nerve stumps prolong the survival of the end organ, such as the taste bud of the Mongolian gerbil (Meriones unguiculatus). Recently, we demonstrated a linear relationship between the length of the IXth nerve stump remaining attached to the gerbil's tongue and the time for taste response amplitudes to fall to 50% of their control magnitudes (Oakley et al., Brain Research 194:213-218, 1980). Perhaps axon stump length is responsible, but in such studies transections are necessarily made at different sites along the nerve trunk. Hence, differential effects might result from the changing axonal branching patterns or from greater disruption of the local blood supply when making nerve exposures nearer to the end organ. We lengthened the gerbil's IXth nerve by causing the axons to regenerate through a nerve splice (a segment of the superior laryngeal nerve). The experimentally lengthened nerves were about 50% longer that the control regenerated IXth nerves. In assessing nerve stump length dependency, both the control and experimental nerves were transected at the same location on the proximal portion of the IXth nerve. The peak magnitudes of the summated taste responses declined at a rate exactly predicted by the earlier nerve stump length dependency study with normal IXth nerves. These experimentally lengthened nerves reformed similar numbers of taste buds relative to control regenerated IXth nerves. We conclude that the trophic features of taste axons are indeed dependent upon axon length and not upon factors uncontrolled in designs which transect at different locations. Additionally, the extra burden of maintaining longer axons does not degrade the nerve's trophic performance in the maintenance of taste buds. Supported in part by NIH Grant NS-07072.

244.11 REGULATION OF CHEMOSENSITIVITY IN CULTURED CHICK CILIARY GANGLION NEURONS. Jeremy B. Tuttle, Physiology Section, Biological Sciences Group, The Univ. of Connecticut, Storrs, CT 06268.

Transmission through the intact ciliary ganglion operates via ganglionic nicotinic ACh receptors. Neurons from embryonic ganglia lose responsiveness to iontophoretically applied ACh during the initial week or so of cell culture. However, ganglionic neurons co-cultured with myotubes rapidly establish neuromuscular transmission and retain this chemosensitivity to ACh (Crean et al, 1982, J. Physiol. 33:87-108). Myotube membrane remnants, prepared by lysis of myotube cultures in water, also support the retention of neuronal ACh sensitivity (Tuttle, 1983, Science, in press). Neuronal responsiveness to other putative neurotransmitters was examined under conditions where nicotinic ACh sensitivity was either retained or had been lost.

Test compounds were dissolved in buffered saline (pH 7.4) and delivered onto cultured neurons by pressure ejection from 2-5 um pipets during intracellular recording. Thus, the extremes of pH and the restricted focal application of iontophoresis were avoided. ACh: (100 uM) Neurons insensitive to iontophoretically applied ACh often responded to ACh delivered by pressure-ejection with a slight very slow hyperpolarization. Current passage through the recording pipet suggested an increase in input resistance accompanied the response. Cells sensitive to iontophoretic ACh responded to pressure ejection with a large rapid depolarization and reduced input resistance. GABA: (1mM) (γ-Amino-n-butyric acid) All neurons tested responded to GABA, regardless of their sensitivity to ACh. If KCl filled the recording pipet, the response was a depolarization with a reversal potential of -30 to -45 mV, whereas the response was hyperpolarizing with a more negative reversal potential when K^+-acetate was used. In either case, input resistance fell during the response. L-Glutamic acid: (1mM) No responses were recorded to glutamate. Leucine Enkephalin: (100 uM) Slow 2-5 mV hyperpolarization resulted from ejection of Leu-enkephalin, accompanied by an increase in resistance on both neurons that had lost nicotinic ACh responses and those that retained sensitivity.

These results suggest the loss of active receptors of a single type (nicotinic ganglionic) is not accompanied by a general loss of responsiveness to other compounds. However, because the loss of nicotinic sensitivity to ACh is rapid in this culture system, longer-term studies are required before the totally independent regulation of transmitter sensitivities can be demonstrated.

Supported by the U.S. Army Research Office, NIH NS 10338 and NS 55402 (RCDA).

244.12 KINETIC CHARACTERISTICS AND MOLECULAR WEIGHT OF ISOLATED NERVE GROWTH FACTOR RECEPTORS. R. W. Stach and J. R. Perez-Polo. Dept. of Human Biol. Chem. & Genet. and The Marine Biomed. Inst., The University of Texas Medical Branch, Galveston, TX 77550.

The nerve growth factor protein (NGF) acts as a classical neuronotrophic factor on vertebrate embryonic sensory neurons and sympathetic neurons at all stages of development. Even though the mechanism of action of NGF at the molecular level is not fully understood, it is known that responsive cells bind NGF at cell surface membrane receptors. There are two different receptors on sensory and sympathetic neurons as judged by kinetic and Rosenthal analyses. These two receptors have equilibrium dissociation constants of approximately 10^{-11} M for the high affinity receptor (type I) and approximately 10^{-9} M for the low affinity receptor (type II). Following isolation of the NGF receptor (NGFR) from embryonic sensory neurons, equilibrium binding studies demonstrated two different equilibrium dissociation constants. Those equilibrium dissociation constants were identical to those that have been determined on whole cells. Receptors from two other cell types (rat pheochromocytoma cells, PC12, and human neuroblastoma cells, LAN-1, which respond to NGF by extending neurites) were also isolated. Equilibrium binding data demonstrated two different equilibrium dissociation constants. These constants were not different from those obtained for the receptors isolated from embryonic sensory neurons. The molecular weights determined for the receptors isolated from embryonic sensory ganglionic cells and PC12 cells were similar. Each had receptor species with molecular weights in the 100,000 to 130,000 range and one or two species in the 200,000 range. It is interesting that both cells requiring NGF for survival, DRG, as well as cells that do not require NGF for survival, PC12 and LAN-1, display similar populations of NGF receptors as determined by kinetic and structural analyses. From kinetic analyses on isolated receptors from embryonic sensory neurons, association and dissociation rate constants were obtained. The association rate constant, determined at 23°, is approximately 1×10^7 M^{-1} sec^{-1} which is identical to that observed on whole cells. There are two different dissociation rates observed. A fast dissociation rate ($k = 3 \times 10^{-2}$ sec^{-1}) and a slow dissociation rate ($k = 2 \times 10^{-4}$ sec^{-1}). The pH binding profile for the two receptors is also different. For the type I receptor, binding displayed a bell shaped response as a function of pH with maximum binding at pH values 7.0-7.9. For the type II receptor, maximum binding was obtained at pH 3.5 and decreased to a constant level at higher pH values. Our evidence is consistent with the hypothesis that there are two different receptors for NGF on these different responsive cells.

Supported by NIH grants NS12325 and NS18708 and The Robert A. Welch Foundation grant H698.

244.13 ENZYME ACTIVITIES IN SINGLE FIBERS OF NEWLY FORMED MOTOR UNITS FOLLOWING NERVE CRUSH. W. R. Turk*, P. M. Nemeth, Dept. of Neurology, Washington Univ. Sch. Med., St. Louis, MO 63110

Muscle fibers within motor units have been shown to have quantitatively identical activities of selected energy-related enzymes (Nemeth et al., J. Physiol. 311, 1981), suggesting that metabolic characteristics of muscle are mediated by the specific neural input. Innervation of muscle fibers can be experimentally altered by lesions of the motor nerve. Collateral sprouting of regenerating motor axons results in a reorganization of the motor unit detected histochemically by the presence of fiber type grouping (Karpati & Engel, Neurol. 18, 1968). We have studied whether enzyme levels are quantitatively similar in fibers newly innervated by a common nerve.

The peroneal nerve of a rat was crushed, producing a motor deficit lasting 4 weeks, and the tibialis anterior muscle was removed five weeks after recovery. Myosin ATPase staining at pH 4.5 was used to demonstrate areas of fiber type grouping and to determine fiber types. Biochemical assays for lactate dehydrogenase (LDH) and adenylokinase (AK) were performed on individual fibers of newly formed motor units and on randomly selected fibers.

FIBER TYPE		n	LDH*		AK*	
I	Motor Unit A	14	29.06 ± 1.46	5.0%	46.75 ± 2.60	5.6%
	Motor Unit B	5	25.95 ± 1.52	5.9%	43.16 ± 0.99	2.3%
	Motor Unit C	6	24.48 ± 2.07	8.5%	30.04 ± 3.33	11.1%
	Random	4	31.69 ± 4.57	14.4%	41.20 ± 1.93	3.4%
	Total	29	27.94 ± 3.20	11.5%	41.83 ± 6.85	16.4%
IIa	Motor Unit	12	30.44 ± 1.74	5.7%	52.99 ± 4.21	7.9%
	Random	6	37.57 ± 4.80	12.8%	49.26 ± 4.48	9.1%
	Total	18	32.82 ± 4.55	13.8%	51.75 ± 4.54	8.8%
IIb	Motor Unit	9	61.11 ± 4.63	7.6%	85.58 ± 2.83	3.3%
	Random	12	64.79 ±11.86	18.3%	81.93 ±10.62	13.0%
	Total	21	63.21 ± 9.75	15.0%	82.91 ± 8.05	9.7%

*Enzyme activities are moles/kg dry wt/hr from duplicate samples expressed as mean ± S.D. and coefficient of variation for the fiber group (n=number of fibers).

The results shows that both enzymes are invariable within most of the new motor units, at the level of experimental error of the method (determined by multiple measures on the same fiber to be 5.8%). The coefficients of variations are significantly less within motor units than among fibers of the same histochemical type, either selected at random or belonging to other motor units. Thus, reinnervation following nerve crush produces new motor units composed of quantitatively similar muscle fibers for LDH and AK. This finding illustrates the marked influence of the motorneuron in directing metabolic plasticity of skeletal muscle fibers.

244.14 SURVIVAL NEURITOGENESIS AND CHOLINERGIC DEVELOPMENT OF ENRICHED CHICK MOTONEURONS GROWN ON HYDRATED COLLAGEN GELS. T.P. Flanigan*, J.G. Dickson* and F.S. Walsh*.(SPON: J. Kenimer). Institute of Neurology, Queen Square, London, U.K.

Considerable evidence has accumulated from in vivo and in vitro studies to suggest that the developmental and regenerative patterns of spinal motoneurons can be influenced by specific cellular and humoral interactions with target muscle fibers. Recently, Schnaar and Schaffner (J. Neurosci. 1, 204, 1981) described a motoneuron-enriched cell fraction (Fraction 1) prepared from 6-day chick embryo spinal cord whose survival in vitro was dependant upon co-culture with muscle cells or the supply of muscle-conditioned culture medium. We have further examined the cell type composition and skeletal muscle dependency of this motoneuron-enriched cell fraction in primary culture.

As previously described, the survival of Fraction 1 cells when cultured on polylysine- or collagen-coated substrata required the presence of myotube-conditioned medium in addition to standard medium supplements (10% horse serum, 10% fetal calf serum, 2% chick embryo extract). However, when seeded onto 3-dimensional hydrated collagen gels, a 75% survival of Fraction 1 cells and the formation of an extensive 3-dimensional neurite lattice was obtained after 7 days in vitro, even in the absence of myotube-conditioned medium. Cells present in these cultures were typically large with granular cytoplasm, distinct nuclei, and neurites extending up to several millimeters into and through the collagen network. Immunofluorescence staining analysis using monoclonal antibody specific for neurofilament protein showed that the cell type composition of cultures was stable up to 7 days in vitro with greater than 99% of cells supporting neurofilament-containing cellular processes. After 14 days in culture however, a number of colonies of proliferating non-neuronal cells were observed to occur at low frequency ($1:10^5$). In addition to cell survival, growth of Fraction 1 cells on the collagen gels resulted in a modest, 2.5-fold increase in the specific activity of choline acetyltransferase over 7 days in culture. This increase is comparable to that obtained during co-culture of Fraction 1 cells with chick myotubes.

The results indicate that the motoneuron enriched cell fraction does not require muscle-conditioned medium for cell survival and neurite outgrowth and, that if operating, any putative muscle-derived motoneuron survival factors must be present in adequate supply in 2% chick embryo extract. It remains however to be determined whether enhanced neurite outgrowth or quantitative changes in cholinergic properties will be produced in response to co-culture with muscle cells or exposure to muscle-conditioned medium.

244.15 A SENSITIVE PERIOD EXISTS FOR THE NEURAL INDUCTION OF CIRCUMVALLATE TASTE BUDS. M.A. Hosley and B. Oakley, Department of Zoology, Neuroscience Lab. Building, Ann Arbor, MI 48109.

The rat has a single midline circumvallate papilla which, at 90 days, contains 625±83 taste buds innervated by the IXth nerves. Removal of both IXth nerves results in the complete loss of taste buds. After unilateral avulsion of the IXth nerve 79% of the taste buds remain (496±54) because of bilateral overlap of the IXth nerves. In our experiments we investigated the role of the nerve supply upon the developmental formation of these taste buds. The right IXth nerve was removed at 3 days post-partum and circumvallate taste buds counted in operated and control rats at ages 5 to 90 days. By 90 days a stable value of 230±36 taste buds was present. These taste buds are widely distributed in the circumvallate papilla indicating that the intact IXth nerve had access to all areas of this gustatory epithelium. When both nerves were crushed at 3 days, 144±68 taste buds were formed by 90 days as a result of bilateral reinnervation. Alternatively, if at 3 days, the right IXth nerve was removed and the left IXth nerve was crushed, only 25±27 taste buds form by 90 days in response to the unilateral reinnervation. Over ages 0 to 30 days the greater the delay in unilateral removal of the IXth nerve, the greater the number of taste buds that eventually form e.g., 474±38 taste buds formed in animals operated on at 30 days and sacrificed at 90 days. We propose an early sensitive period extending to age 30 days in which nerve fibers and taste bud precursors must interact to ensure the formation of normal numbers of taste buds. Experiments with crushed nerves indicate that reinnervation after the critical period is ineffective from which we conclude that the effects, in these circumstances, of early denervation are irreversible.

Supported in part by NIH Grant NS-07072 and a Grant from The University of Michigan Rackham School of Graduate Studies.

244.16 TARGET INFLUENCES UPON TRANSMITTER SYNTHESIS IN CULTURED CHICK CILIARY GANGLION NEURONS. D. Bruce Gray and Jeremy B. Tuttle, Physiology Section, Biological Sciences Group, The University of Connecticut, Storrs, CT 06268

Primary cultures of chick ciliary ganglion neurons have been established as an in vitro model for studies of neuronal differentiation and its trophic regulation. Recent evidence from this lab has shown that co-culture of chick ciliary ganglion neurons with chick pectoral muscle increases acetylcholine (ACh) synthesis both at basal levels and after K^+-induced depolarization as compared to neurons cultured alone. Synthetic responsiveness to depolarization is a characteristic of mature ciliary neuromuscular junctions in vivo and may depend upon developmentally relevant target interaction.

Initially, the culture system was characterized metabolically by examining choline uptake, choline metabolism and ACh synthesis, measured by incorporation of ^{3}H-choline. Although large amounts of non-specific, Na^+-independent uptake of ^{3}H-choline occurred in cultures at low choline concentrations, Na^+-dependent ACh synthesis clearly predominated over Na^+-independent synthesis at choline concentrations of less than 10 uM with a Km at approximately 1 um and a Vmax at 2 fm ACh synthesized/hour/cell. Na^+-independent synthesis had a Km of >50 uM choline and a Vmax of >6 fm ACh syn/hour/cell. In our assay, synthesis and degradation appeared to reach equilibrium after 30 minutes in ^{3}H-choline, in both control and 0 Na^+ incubations.

Preliminary experiments show that co-culture of neurons upon a substrate of lysed myotube membranes mimic the ability of live myotubes to stimulate ACh synthesis following 10 min preincubation of cultures in high K^+ (55 mM). Lysed myotube membranes were prepared by incubation of 5-day cultures of 11 day chick pectoral muscle on collagen coated microwells, in distilled H_2O for 2 hours followed by 3 washes in normal growth medium. Neurons plated upon lysed myotube membranes increased ACh synthesis by 76% in response to high K^+ preincubation, as opposed to a <40% increase in cultures of neurons alone.

Live muscle in co-culture (Crean et al, 1982, J. Physiol. 33: 87-108) and the same lysed membrane preparation (Tuttle, 1983, Science, in press) also support neuronal chemosensitivity to ACh. Thus, these results implicate contact with target membranes as an important aspect of neuronal differentiation. Both the ability to rapidly replenish transmitter stores in response to demand and the responsiveness to transmitter of these cultured neurons are profoundly influenced by contact with target tissue membranes.

Supported by the U.S. Army Research Office; NIH NS 10338; NS 55402 (RCDA) and the Univ. of Conn. Research Foundation.

244.17 CONDITIONED MEDIA AND CYCLIC AMP INFLUENCE NEURONAL SURVIVAL DURING ELECTRICAL BLOCKADE. D.E. Brenneman, S. Fitzgerald, P.G. Nelson. (SPON: Elaine A. Neale) Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, Maryland 20205.

The relationship between electrical activity and the action of neuronal survival factors was investigated in dissociated spinal cord cultures. The possible role of cyclic nucleotides in determining neuronal survival during electrical blockade was also studied. Spinal cord-dorsal root ganglia (SC-DRG) cultures were prepared from 12-14 day old fetal mice.

Previous studies have shown that blockade of spontaneous action potentials with tetrodotoxin (TTX) produced a significant decrement in cholinergic development and neuronal survival. Vulnerability of spinal cord neurons to TTX treatment occurred during a limited period in development (days 7-21 in culture). We now report that conditioned media (CM) obtained before or after but not during the critical period produced a dose-dependent protection for neurons grown in the presence of TTX during the vulnerable period. This sparing effect of the CM was determined from neuronal cell counts, ^{125}I-tetanus toxin fixation, and choline acetyltransferase measurements. The protective action present in CM was shown to be TTX-sensitive; i.e., the presence of 10^{-7} M TTX during the collection period prevented the protective activity of the CM. This lack of protective action was not attributable to a loss of donnor neurons during the collection period. Cell counts revealed a 20-25% decrease in neurons when TTX was applied during days 10-15 in culture. Application of TTX plus CM from young cells produced a 25% increase in the number of neurons as compared to control cultures. CM alone produced no change in neuronal cell counts from that of controls. Thus, electrical blockade in the presence of CM from young cells produced an increase in neuron survival.

Supplementation of culture media with dibutyryl cyclic AMP and TTX produed a dose-dependent increase in ^{125}I-tetanus toxin fixation as compared to cells treated with TTX alone during the critical period. Similar effects were observed with 8-bromo cyclic AMP. Addition of 8-bromo cyclic GMP had no effect on the TTX-mediated decrease in ^{125}I-tetanus toxin. These studies suggest that increases in cyclic AMP may be involved in mediating the action of some survival factors during development.

These data indicate that neuronal survival factors are released during different periods in development. The release of some of these survival factors is dependent on electrical activity. The competition for or utilization of these factors may depend on the electrical state of target neurons. Cyclic AMP may also play a role in mediating the survival response. It is concluded that electrical activity has multiple roles in determining the survival of neurons during epigenetic development of the CNS.

244.18 DISSOCIATED NEURONS FROM ADULT RAT SUPERIOR CERVICAL GANGLION SHOW REDUCED NGF REQUIREMENTS IN CULTURE. M.I. Johnson, Dept. Anatomy & Neurobiol., Washington Univ. Sch. Med., St. Louis, MO.

The principal neurons from the superior cervical ganglion of adult rats were dissociated into culture using a previously described method (J. Neurophysiol. 42:1410, 1979). For experiments on acute survival the neurons were grown on supplemented medium containing human placental serum and embryo extract with or without NGF. After 10 to 14 days those cultures on medium without NGF were returned to NGF containing feed and one week later neuronal counts made. In 3 separate series neurons initially seeded in medium without added NGF showed greater than 80% survival compared to the neurons in fully supplemented feed. In contast neurons dissociated from perinatal rats and treated similarly showed only 0.9% survival. Established cultures of adult neurons were withdrawn from NGF and studied using neuronal counts and measurements of neuronal diameters. Neuronal counts decreased to approximately 50-60% of control cultures over 7-10 days of NGF deprivation; if NGF was added back neuronal counts returned almost to control levels. Neuronal diameter showed a rapid decrease to approximately 65% of control neurons but again increased to almost control values with the return of NGF in the medium. Sequential photographs of identified neurons in the living cultures showed morphological changes consistent with chromatolytic reaction upon NGF withdrawal and some neurons became unrecognizable by criteria established for the neuronal counts. It is concluded that SCG neurons from adult rats are less dependent on NGF for survival and maintenance than those from perinatal rats.

244.19 DEVELOPMENT OF OPIATE RECEPTORS ON RAT DORSAL ROOT GANGLIA IN TISSUE CULTURE: ROLE OF GLIAL CELLS. Samir F. Atweh and Riley Yu. Department of Neurology, University of Chicago, Chicago, IL 60637.

Opiate receptors are known to develop with time both in brains of fetal and new born rats (Coyle and Pert; Neuropharmacology 15: 555, 1976) and in Neuroblastoma x Glioma hybrid (NG) cells in tissue culture (Atweh; Soc. Neurosci. Abst. 7: 433, 1981). In the NG cells we previously demonstrated that a secreted factor, that is probably derived from glial cells, stimulates the formation and/or expression of opiate receptors (Atweh and Arnason; Soc. Neurosci. Abst. 8:187, 1982). In this study we show that opiate receptors develop on isolated rat dorsal root garglia (DRG) in vitro, and that non-neuronal supportive cells are important for the development of the receptors.

DRG's were carefully dissected from the cervical region of 17 day old embryos of Haltzman albino rats. One or two DRG's were explanted on a 22mm round coverglass coated with 50µl reconstituted rat-tail collagen and maintained in culture medium consisting of Eagle's minimal essential medium, human placental cord serum and chicken embryo extract. The medium was supplemented with glucose and glutamine and the cultures were maintained for 2-8weeks in a CO_2 incubator with 95% humidity. Some cultures were treated with 5-flourodeoxyuridine (10^{-5}M) for 48hrs. followed with cytosine arabinoside (10^{-5}M) for 48hrs. to control proliferating non-neuronal cells. This treatment was initiated one week after explantation and was repeated twice. At various intervals after explantation the attached DRG's were washed two times with physiological saline and incubated with ^{3}H-Etorphine (1nM,35Ci/mm) in 150mM-Tris buffer at room temperature. The slides were then washed twice in ice-cold buffer. The DRG's were then scraped off the slide, digested with tissue solubilizer and counted for radioactivity. Non-specific binding was determined in the presence of excess Naloxone (10^{-4}M) in the incubating buffer.

Specific etorphine binding (approximately 50% of total binding) occurred only on growing DRG's, and not on the collagen coating. Specific binding per mg DRG protein was negligible 2 days after explanation but gradually increased up to 4weeks in culture and was maintained up to 8weeks. The treated ganglia showed normal neuronal outgrowths, but they lacked supportive cells and there was no myelination. In these ganglia there was 84% and 79% reduction in etorphine binding at 4weeks and 8weeks, respectively, as compared to untreated ganglia. This system might be useful in studying some of the factors that regulate the expression of opiate receptors.

MORPHOGENESIS AND PATTERN FORMATION

245.1 EFFECTS OF NEURAL CREST DELETIONS ON THE SEGMENTAL PATTERN OF SKIN SENSORY INNERVATION IN EMBRYONIC CHICK HINDLIMB. S. A. Scott. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

Studies of normal development have shown that the segmental pattern of skin sensory innervation in the chick hindlimb is established in a precise, orderly manner (Honig, J. Physiol. 1982; Scott, J. Physiol. 1982), but have revealed little about the mechanisms that determine the axonal projection pathways and the location and size of dermatomes. To test whether competition among DRG axons plays a role in the development of skin innervation patterns, the DRGs that innervate skin on the anterior thigh and shank (T7-LS1) or on the distal shank and foot (LS4-6) were deleted by removing the parent neural crest region in St15-17 embryos, and the dermatomes and/or axonal projections of the remaining DRGs were mapped at St27-41.

In "mature" St37-41 embryos dermatomes of the remaining DRGs had expanded into denervated skin regions, partly but not completely replacing the lost innervation; the extent of the enlargement depended on the amount of skin denervated. Enlarged dermatomes were observed in embryos as young as St30, soon after skin innervation is established, indicating that axons ignored their usual dermatome borders, and grew without detectable delay into inappropriate, denervated skin regions.

Axonal projections were mapped physiologically in mature embryos, and with HRP injections in St27-30 embryos. In both groups the cutaneous nerves usually supplied by the deleted DRGs were missing, and axons from intact DRGs were seldom found in aberrant pathways. In the mature embryos, however, the distribution of DRG axons within their usual pathways was altered, projections from distant DRGs being larger than normal in the pathways near those of the deleted DRGs . These changes in axonal projections were apparently made at the expense of the intact DRGs' other projections, which occasionally were reduced in size or missing, as if all skin sensory axons shifted their projections into pathways near the deleted ones. Such altered projection patterns were seen in embryos as young as St27-30, before the bulk of cell death, and thus reflect a change in the initial outgrowth of axons. These changes in the relative distribution of axons may have been further enhanced in mature embryos by the selective rescue from cell death of neurons that innervated the denervated skin regions.

Together these results suggest that during normal development competition among ingrowing axons may be important in establishing both the borders between dermatomes, as well as the distribution of DRG axons among their projection pathways. (Supported by NIH grant NS16067 and an Alfred P. Sloan Foundation Fellowship).

245.2 DEVELOPMENT OF SUPRASPINAL INPUT INTO THE TAIL SPINAL CORD OF XENOPUS. R. H. Nordlander and T. J. Ryba*. Dept. Oral Biology, Case Western Reserve University, Cleveland, OH 44106

The earliest descending axons in the developing tail spinal cord of Xenopus are of local origin (Nordlander, R.H. Soc. Neurosci. Abst. 8: 635, 1982). The cell bodies belonging to these axons were identified using horseradish peroxidase (HRP) applied to the tail spinal cord near its tip. Following application to more rostral levels of the tail cord, HRP also appears in supraspinal neurons. It is these cells and the time course of the entry of their axons into the tail which will be described here.

Recrystalized HRP was applied to the spinal cords of Xenopus of stages 32-48 at the level of the 17th myotome. After 5 to 48 hr. of incubation, specimens were fixed, reacted according to the method of Hanker, et al. (Histochem. J., 9: 789, 1977), and processed for light microscopic viewing as wholemounts, vibratome sections or 5 µ plastic sections in transverse or horizontal planes.

Labeled axons appeared in two major fascicle groups extending from the application site to the brain. One, dorsolateral in the spinal cord, fans laterally upon entering the brain continuing rostrally as far as the cerebellum. It contains ascending processes of the Rohon-Beard cells of the spinal cord. The second group of labeled fascicles is located ventrolaterally in the spinal cord and continues ventrally in the brainstem. It is into these bundles that the earliest descending brain neurons feed their axons.

Early brain cells labeling under these conditions include reticular, raphe, and vestibular cells in the hindbrain and a cluster of mesencephalic neurons. Most prominent and earliest of the reticular neurons to appear are the paired Mauthner cells which first label at stage 37 and consistently thereafter. Other hindbrain reticular cells are added gradually over the period of this study. They are grouped in three clusters probably corresponding to the rostral, middle and caudal groups described in larval zebrafish (Kimmel, C.B., et al, J. Comp. Neurol., 205: 112, 1982) and the nuclei reticularis superior, medius, and inferior of older Xenopus tadpoles (ten Donkelaar, H.J. and R. de Boer-van Huizen, Anat. Embryol., 163: 461, 1982). In early Xenopus larvae the caudal (inferior) group is the most conspicuous. Medial to these cells and appearing at stage 40 are the raphe neurons. Vestibular cells (ventral nucleus of VIII) are arranged in a column along the dorsolateral fascicle of Rohon-Beard axons. The first of these to label, at stage 39, are those closest to the Mauthner cells. Labeled cells in the mesencephalon are located ventromedially near the junction of mid- and hindbrains and are considered to belong to the nucleus of the medial longitudinal fasciculus.

245.3 CHANGES IN CELL SHAPES DURING BENDING OF THE FUTURE BRAIN REGION OF THE AVIAN NEURAL PLATE. G.C. Schoenwolf and M.V. Franks*. Dept. of Anatomy, Univ. of Utah Sch. of Med., Salt Lake City, UT 84132.

It is widely believed that changes in cell shapes play an important role in the bending or folding of epithelial sheets. However, few studies have actually examined cell shapes in bending epithelial sheets. We have examined neuroepithelial cell shapes during bending of the neural plate at two craniocaudal levels of the future brain (mesencephalon and rhombencephalon) in stage 4-9 chick embryos. During elevation of the neural folds, bending of the neural plate is restricted to the midline, supranotochordal area of the neural plate. Likewise, bending is restricted to paired dorsolateral areas of the plate during convergence of the neural folds. In the future mesencephalon, the neural plate was subdivided into a supranotochordal area and paired lateral areas to examine cell shapes during elevation of the neural folds. In the future rhombencephalon, each lateral area of the plate was further subdivided into dorsolateral and intermediate areas to examine cell shapes during convergence of the neural folds (the dorsolateral area contains a well-defined locus of bending during convergence of the neural folds, whereas the intermediate area is located between the supranotochordal and the dorsolateral areas of bending). Neuroepithelial cells in all areas of the neural plate had four characteristic shapes: flask like (tapering apically), inverted flask like (tapering basally), sphere like (cells in the M phase of the mitotic cycle) and spindle like (tapering both apically and basally). The percentages of sphere-like and inverted flask-like cells remained statistically unchanged during bending of the neural plate. In contrast, the percentages of spindle-like and flask-like cells changed markedly in certain areas of the plate as follows. In the supranotochordal region of the future mesencephalon, spindle-like cells decreased sharply during elevation (from 69 to 28%), while flask-like cells increased (from 27 to 70%). Only slight changes occurred laterally (spindle-like cells decreased from 69 to 66%, while flask-like cells increased from 24 to 26%). In the dorsolateral region of the rhombencephalon, 57% of the neuroepithelial cells were flask like during convergence of the neural folds, while 39% of the cells were spindle like. In the intermediate area of the rhombencephalon, 34% of the cells were flask like and 61% were spindle like. These results demonstrate that many of the cells that are restricted to loci of bending during elevation and convergence of the neural folds become wedge shaped, whereas little change in cell shapes occurs in adjacent areas of the neuroepithelium. Whether these changes in cell shapes constitute a driving force for bending of the neural plate remains to be investigated. Supported by NIH grants nos. HD 15231 and NS 18112.

245.4 SOMA POSITION CORRELATES WITH TIME OF DEVELOPMENT IN TWO GROUPS OF IDENTIFIED NEURON TYPES OF THE LARVAL ZEBRAFISH (Brachydanio rerio). B. Mendelson* (SPON: D. P. Kimble). Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

Five types of identified reticulospinal neurons were studied in Brachydanio rerio in order to learn how particular morphological features may be correlated with time of development. These cells fall into two broad groups based on their morphology (Kimmel, C.B. et al., J. Comp. Neurol., 205:112, 1982). The first group includes the Mauthner neurons plus the MiD1 and MiD2 cell types. These neurons all have large somata located dorsally in the hindbrain reticular formation and axons which decussate to descend in the contralateral dorsal medial longitudinal fascicle (mlf). The second group includes the MiV1 and MiV2 cells. These hindbrain reticular neurons all have ventrally located somata and axons which descend in the ipsilateral ventral mlf. I proposed that the morphological differences observed between these two groups of cells are in part due to differences in the times of development of these identified neurons.

The time of origin of these neurons was determined by injecting ^{3}H-thymidine into the embryo. Neurons of the dorsal group were seen to change from 100% labeled to 0% labeled after injection between 7 and 12.5 hrs after fertilization. The ventral group of cells were always found to be labeled at the injection times so far tested (through 13 hrs post-fertilization). This shows that the birthday of the dorsal cells is earlier than that of the ventral cells.

The temporal order of axon outgrowth displayed by these cells was also studied, by finding the earliest developmental times that the neurons could be retrogradely filled with HRP from a lesion located about 200 μm caudal to their somata. The dorsal cells could be first labeled with HRP 20 hrs after fertilization while the ventral cells were first labeled 12 hrs later. If these different reticulospinal axons elongate at the same rate, then the dorsal cells initiate axon outgrowth before those of the ventral cells.

The differences in morphology displayed by the two groups of reticulospinal cells are correlated with differences in times of development of these neurons. Reticulospinal cells with dorsally located somata and axons which decussate leave the cell cycle and initiate axon outgrowth before reticulospinal neurons which display ventral somatic position and ipsilaterally projecting axons. The observed temporal order of developmental events may, in part, specify the somatic positions and modes of axonal projection. (Supported by NIH grant NS 17963 and #5 T32 GM07257-07.)

245.5 MORPHOLOGY AND DEVELOPMENT OF MOTONEURONS IN THE ZEBRAFISH SPINAL CORD. P.Z. Myers* (SPON: C. B. Kimmel). Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

HRP injections into the axial muscles of the larval zebrafish have revealed two distinct classes of spinal motoneurons. The development of these two neuronal types, primary and secondary motoneurons, has been followed with both the light and electron microscope in order to explore the temporal relationships between their differentiation and the growth of the Mauthner axon into the spinal cord.

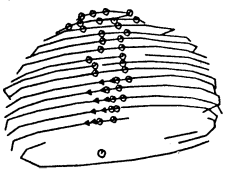
The primary motoneurons are large, distinctive cells situated laterally in the cord. They have thick, tapered ventral neurites which always pass medially and adjacent to the Mauthner axon, from which they receive a synapse before they exit the cord at the ventral root. Secondary motoneurons are much smaller cells which lie in a ventrolateral column. Their peripheral process always passes laterally and without any consistent association or apparent synapse with the Mauthner axon before entering the ventral root. I have hypothesized that the timing of development in the spinal cord may play a role in defining the primary motoneurons as the Mauthner axon's specific target.

Primary motoneurons in a given segment can be labelled from a peripheral lesion three hours before the Mauthner axon growth cone has reached that segment. I have not been able to label secondary motor neurons, however, until three or four hours after the Mauthner axon has grown by. New neurites appear to be added to the growing spinal cord laterally, and the relative positions of the axons of the primary and secondary motoneurons and the Mauthner axon also imply that they were added in the order primary motoneuron : Mauthner axon : secondary motoneuron.

These data indicate that there is a precise sequence of neurite growth in the spinal cord. I suggest that the Mauthner axon selects the primary motoneurons as its targets because they, specifically, have developed neurites which could serve as targets for synaptogenesis by the time they are contacted by the Mauthner growth cone. (Supported by NIH grant NS17963.)

245.6 COMPUTER AIDED RECONSTRUCTION USED TO VISUALIZE THE 3-D SHAPE OF CHIMERIC XENOPUS EYES. G.B. Stock*, K. Conway, and R.K. Hunt*. Jenkins Dept. Biophysics, The Johns Hopkins Univ., Balt. MD 21218.

When minute eyebud grafts are exchanged between normal and albino embryos, chimeric froglets with sectors of marked eye tissue develop. Analysis of the shapes of such regions provides detailed information about the growth of the eye and was accomplished both by photography of live, anesthetized froglets and by careful examination of histological sections of preserved eyetissue. Using a camera lucida attachment to a compound microscope, tracings were made from sections to obtain the contours of the pigment retinal epithelium (PRE), and also contours of selected extraocular landmarks, which were used to align and orient consecutively traced sections. Contours of marked PRE were also traced and identified. These contours were then converted to a sequence of representative cartesian coordinates using a digitizing tablet connected to an IBM 4341 via a CRT terminal. A Fortran program managed the data input so as to associate with each contour record an identifier of tissue type and a section number. This information, coupled with the section thickness and a magnification factor from the tracing optics, provides an accurate representation of the 3-D geometry of marked tissue in the eye and is readily manipulated during analysis. The data for each eye was stored on disk and archived on tape in a suitably labelled file. Eye features were visualized by interacting with a sophisticated Fortran program that created perspective images of the eye from any desired viewing angle. Additional manipulation was possible by viewing particular tissue types, changing the scale, creating stereo viewing pairs, and controlling the display of hidden lines. Displays could be routed to a variety of output devices, including the CRT, Tektronics CRT, Calcomp plotter, or a daisy wheel printer. The Fortran programs are easy to use and are available on request. The plotted images employed a variety of graphic symbols to highlight pigmented and non-pigmented regions of tissue. To ensure that no gross distortion had occurred as a result of the analytical procedures employed, a number of these images were compared to corresponding photos taken prior to fixation. This 3-D reconstruction technique is quite flexible yet inexpensive, is clearly useful in analyzing anatomical features, and is currently being applied to other systems, such as peptidergic neurons in the hypothalamus, and regions of cell death and damage in lesioned spinal cords. Also, once an eye is digitized, further numerical analysis of its geometry is feasible. We thank NIH (GM-07231, NS-14807), NSF (PCM 77-26987), and the Dean's computing fund at Johns Hopkins.



245.7 CLONAL GROWTH PATTERNS IN GENETICALLY CHIMERIC EYES OF XENOPUS. J.S. Cohen* and R.K. Hunt* (SPON: W.M. Cowan) Biophysics Dept., Johns Hopkins Univ., Baltimore, MD 21218, and The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

The frog eye is an attractive model system for studies of clonal growth in organogenesis. A germinal ring, interposed between the retinal epithelia and iris, adds annuli of new cells to the margin of the pigment epithelium (PRE) and neural retina, such that the oldest cells lie at the back of the eye and the youngest at the front. Moreover, a ventral-to-dorsal gradient in cell accretion rates is demonstrable by ^{3}H-thymidine autoradiography, from metamorphic climax (Jacobson, Brain Res. 103:541, 1976). When small wedges of eye rudiment are surgically introduced from wild-type (or l-nu or 4n) pigmented donor embryos into albino (albP/albP) host anlagen (at stages 30-34), the marked wedge: (i) heals to form a sector of marked cells in the PRE, neural retina and iris of the early larval eye; (ii) the sector elongates radially during larval growth, by the addition of marked cells to its distal end, and (iii) can be analyzed for variations in sector shape, fine cell patterns at graft/host sector boundaries, and fluctuations in growth over later larval stages (Conway et al., 1980, Curr. Tops. Develop. Biol. 15:217). We have serially photographed the developing black/white sector pattern in 60 chimeric larvae, bearing wedge-grafts at dorsal or at the abutting posterior and anterior regions of the anlage - examining the relative decline in cell accretion rates at the dorsum of the metamorphosing eye. Sector patterns were stable during metamorphosis, save for new tissue added to the distal end of the sector, indicating that PRE cells do not move with respect to one another. New growth declined dorsally; marked dorsal sectors often tapered into single cell files; abutting posterior and anterior stem cells and iris veered dorsally, and began to make 'replacement' cell files for the attenuating dorsal files, which maintain continuity of the PRE cell sheet. Serial photography of elongating cell files permitted direct quantitation of mitotic rate and cell death. Mitotic rates varied up to 5-fold between individual cell files in a single eye, and significant variations occasionally occurred within a single growing file over time. Cell deaths were very rare but were easily visualized, and black cell debris was cleared gradually over several days. Although separate lineages for iris, PRE and neural retina are founded in the early larva (Conway et al., op cit.), the replacement cell files in dorsal PRE at metamorphosis arose in association with thin, radial iris fibers. These iris fibers may undergo metaplastic transformation from iris into PRE (Eguchi, 1975, Ciba Symp. 40, 241) or provide 'guidewires' for covert PRE stem cells, and are under study in TEM. Their role may also be more general: split sectors in anterior PRE also showed cell files associated with iris fibers; 4n/albino chimerae showed cell files in neural retina, strikingly similar in form to those in PRE, and continuous with the inner amelanotic layer of the iris.

Supported by NIH Grant GM-07231 and NSF PCM79-03827.

245.8 PARSIMONIOUS REPRESENTATION OF INFORMATION IN LAYERED NEURAL SYSTEMS. R. B. Glassman. Dept. of Psychology, Lake Forest College, Lake Forest, IL 60045.

Modelers must think not only about hypothetical neural networks that can do the behavioral job but about ones that can plausibly be thought to have evolved by small steps, each adaptive, but each random with respect to the cumulative outcome (Darwin, 1859). A corollary of this basic rule is that organisms likely have evolved fairly parsimonious, "cost-efficient" neural facilities for storing information.

Although the ideas of stages of information processing and layered or hierarchial structures have been considered extensively in the literatures of cognitive psychology and artificial intelligence, to my knowledge the point has not yet been made that a certain form of such structuring fits the above corollary. While the connections between levels of a functionally layered neural structure provide instances of organized products of evolution, this form of organization permits an "inexpensive," low degree of ordered complexity within levels. Indeed, the basic task of organizing inputs and outputs in a layered system can be accomplished by subsystems, within each of which the rule of association is simply synaptic spatial summation. To see this, consider a neural model of the cat's ability to orient its face toward a tactile stimulus at any point on the body.

As previously argued, somesthetic spatial orientation might rest equally well on a nonlayered neural foundation of all possible connections between all discriminable areas on the skin (say there are s) and all muscle groups (say there are r), for a total number sr of spatially summating connections. However, a single interposed associative layer, having three subsystems representing the vertical, horizontal, and anterior-posterior dimensions, could accomplish the same task with only 3s+3r spatially summating connections (1982 Abstract 272.13; Physiological Psychology, 1983).

It remains to note that this model is potentially a general one that may be used to describe any three successive layers of input-association-output in a multilevel system. One proviso is that a multitude of input-output relations must lend themselves to categorization according to a relatively few "dimensions," in such a way that each input-output relation is determined by a unique distribution of excitations of the subsystems in the associative layer.

Fine tuning of such systems, which would take the form of adjusting excitatory strengths of the various connections, must take place during ontogeny. Such fine adjustment of "biases" in earlier-developing subsystems must be a prerequisite for the organization of later-developing associative layers (Glassman & Wimsatt in Almli & Finger, Eds., The behavioral biology of early brain damage, in preparation, Academic Press).

245.9 INTRACEREBRAL GRAFTS OF DISSOCIATED CEREBELLAR TISSUE FORM TRILAMINAR CORTICAL STRUCTURES. R.H. Schmidt and R.K. Bhatnagar. Dept. Pharmacolgy, University of Iowa, Iowa City, IA 52242.

A new approach to intracerebral neuron transplantation has recently been described which is based on the stereotaxic injection of fluid suspensions of embryonic donor neurons (Schmidt et al. Brain Res. 218: 347). Because this technique involves the total trypsin dissociation of donor tissues, it was of interest to a) relate the survival of specific neuron types in the grafts to the variable of donor age, and b) study the architecture of the resultant grafts. Cerebellar tissue grafts were ideally suited for such a study.

Suspension grafts were prepared from donor rats ranging in age from embryonic day (ED) 15 to postnatal day 5, and injected into the forebrain of adult hosts. At survival times up to 7 months host brains were processed for cresyl violet and silver fiber staining. Purkinje cells (PC) were clearly discernable in grafts from ED 15-18 (14-30 mm CRL) donors and granule cells (GC) in grafts from all donor ages. Based on the normal proliferative periods for these neuron types it appears that PC do not survive the dissociation if taken more than 48 hours postmitotically. On the other hand the results with GCs show that neuroblasts do survive the dissociation and can proliferate within the adult host for a period of time.

Grafts from ED 16 donors injected into the parenchyma of the cerebral cortex did not develop any architectural organization. However, if the grafts were deposited onto a surface structure, such as the ventricular surface of the hippocampus, the grafted neurons organized into aggregates containing distinctly trilaminar architecture typical of the cerebellar cortex. PCs generally became aligned into monolayers between a cell-sparse molecular layer on the outer surface of the graft and an inner zone of densely aggregated GCs. Typically the width of the molecular layer was equivalent to the width of the GC layer, and in some grafts these zones exceeded 60 μ in thickness. Fiber staining showed that every PC was heavily invested with a basket-cell-like axonal plexus typical of the normal cerebellum. Details of the dendritic and fiber architecture in the molecular layer were not revealed. At survival times of 14-20 days post-transplantation the architecture was typical of the developing postnatal cerebellum with an apparent external granule cell layer, thin molecular and internal GC layers, small PC diameters and a sparse basket cell plexus. The architecture suggests that development within these grafts recapitulates the normal ontogeny of the postnatal cerebellum, with GCs migrating from an external proliferative zone into the internal granular layer. These grafts thus represent a novel model system with which to define the mechanisms of these developmental events. (GM-22365)

245.10 MORPHOLOGY OF SUBSTANTIA NIGRA NEURONS TRANSPLANTED INTO THE LATERAL VENTRICLE OF THE RAT: A GOLGI STUDY. B. Hoffer*, S. Wood*, W. Freed, R. Wyatt and G. Rose*. VMAC, Denver, CO and NIMH, St. Elizabeth's Hospital, Washington, D.C.

Transplantation of fetal substantia nigra into either the lateral ventricle adjacent to, or in a cavity created in the cortex overlying, a dopamine-denervated caudate will reduce or eliminate behavioral deficits caused by the denervation. As an initial step toward determining how this process occurs, we chose to examine the morphology of the transplanted neurons. The anlage of the substantia nigra was obtained from fetal donors, suspended in 10 μl of artificial CSF, and immediately injected into the lateral ventricle of adult (90-150 day old) host rats. Four months prior to transplantation half of the host animals had received a unilateral 6-OHDA lesion of the substantia nigra. Five months after transplantation all animals were sacrificed and the brains processed using the Golgi-Cox method of Van der Loos. Both neuronal and non-neuronal cells were impregnated in the transplants. The neurons could be roughly divided into three groups, based upon cell body size. Small cells (diameter <20μ) had mostly rounded somata, thin dendrites, usually with varicosities and lacking spines, and a generally stellate appearance. Medium cells (diameter 20-36μ) had somata that were either round or ovoid, although occasionally pyramidal or fusiform cell bodies were observed. These cells tended to have a bipolar orientation, with long dendrites having few branches. These dendrites were either spiny or varicose; only rarely were varicosities with spines observed. Large cells (diameter >40μ) had ovoid, fusiform or pyramidal somata, and thick dendrites lacking varicosities or spines except at their extremities. Small cells comprised approximately 11% of the neurons seen, medium cells 72% and large cells 17%. The three cell groups observed have good correspondence to those described for the in situ substantia nigra. However, in other respects the substantia nigra transplants differed from their in situ counterparts. No regional organization was apparent in the transplants; neither was any obvious dendritic orientation observed. In addition, dendritic varicosities were very often seen, perhaps indicating incomplete maturation of the transplanted neurons. Placement of the transplant adjacent to a dopamine-denervated caudate nucleus did not seem to promote either dendritic differentiation or outgrowth. In summary, neurons in transplants of fetal substantia nigra placed in the lateral ventricle of adult rats grow and differentiate into cells morphologically similar to their in situ counterparts. This process apparently does not require the presence of normal afferents to the structure.

245.11 A QUALITATIVE AND QUANTITATIVE STUDY OF MYELINATED AXONS AND GLIAL CELLS IN THE DEVELOPING MOUSE SPINAL CORD. J.C.Zagoren*, H.Nagara* and K.Suzuki. Depts. of Neuroscience and Pathology, Albert Einstein College of Medicine, Bronx, N.Y.10461. Glial and myelin development has been analyzed in dorsal and ventral tracts of mouse spinal cord during the first month of post-natal life. C57Bl mice at 1,3,5,6,7,8,9,13,16,18,24,and 30 days of age were prepared for microscopy by fixation, either immersion or perfusion, in 2.5% glutaraldehyde. Cross sections of cervical (C1-3 and C6-8), thoracic (T4-6) and lumbar (L2-3) cord were obtained from 45 animals. Glial cells were identified by comparing light microscope (LM) and electron microscope (EM) images in successive serial sections. Discrimination between glial cells was difficult, since cells with features of oligodendrocytes sometimes contained filaments typical of astrocytes upon EM examination. Total glial cells were counted at each timepoint and spinal level and found to increase as a function of tract, level and time. A three way analysis of variance was performed to ascertain the degree of significance in the numbers of glial cells and myelinated axons in a rostral to caudal direction, in a dorsal to ventral direction and a difference in numbers with age. The results showed no rostral to caudal difference in either the ventral or dorsal tracts but did show a change with age and an interaction between the dorsal and ventral tracts with days in the cervical, thoracic and lumbar tracts. Numbers of glial cells increased prior to myelinogenesis and decreased following myelin completion. Formation of myelin lamellae was first observed in the lumbar ventral tract at day 1. By day 3, only the lumbar dorsal area remained unmyelinated. At day 5 there was a 65-fold mean increase in myelinated axons and by day 9 mature nodes of Ranvier were seen. In summary, these data show that post-natal changes in glial number and pattern of myelination vary in individual tracts and levels of the spinal cord. The conventional idea that myelination proceeds evenly from caudal to rostral regions therefore may require further qualification.

245.12 A NEUROANATOMICAL CORRELATE OF SCHIZOPHRENIA. J.A. Kovelman and A.B. Scheibel, M.D. Dept. of Anatomy, UCLA Sch. of Med., Los Angeles, CA 90024.

We report a structural anomaly of hippocampal pyramidal cell alignment in the left hippocampi of ten chronic schizophrenics compared with eight age-matched, non-psychotic controls. Under blind conditions, three stations along Ammon's Horn were studied; the interfaces between CA1 and prosubiculum, CA1 and CA2, and CA2 and CA3. These positions were examined at three longitudinal loci: anterior, middle and posterior hippocampus. In order to quantify the cytoarchitectonic disorganization which was found, the angles of orientation of 120 pyramidal cells in each block of Nissl-stained tissue were measured against an ideal axis of reference drawn perpendicular to the ventricular surface at that point. Abnormally positioned pyramidal cells were observed in chronic schizophrenics with some rotated as much as 90° from the expected alignment, whereas neurons from controls showed significantly smaller amounts of deviation. Our results, limited so far to the left hemisphere, indicate that the chronic schizophrenic hippocampus has a significantly greater degree of pyramidal cell disorientation than controls at all three CA interfaces when the longitudinal axis is ignored. The effects of cell disarray are more striking in middle hippocampus and when values from anterior-middle hippocampal tissue are combined. No significant difference was found for any CA locus in posterior hippocampus. Note that abnormal electrical activity previously reported from deep temporal lobe stations in schizophrenic patients is found only in anterior and middle positions.

Studies of normal hippocampal development as well as those of several genetic mutants support our assumption that such cell disarray may occur during prenatal development when neuroblasts migrate and align themselves in primordial hippocampus. The findings of enhanced cell disarray in middle and anterior-mid hippocampus, but not posterior hippocampus are consistent with the concept that different parts of the hippocampus subserve different functions. Since deviations of smaller degree are also found in some non-psychotic patients, the study suggests a histopathological continuum related to behavior and character structure, from the borderline individual to the intractable, long-term psychotic.

(Supported by a grant from Scottish Rite Schizophrenia Research Program, N.M.J., U.S.A.)

245.13 CENTRAL TRIGEMINAL REPRESENTATIONS IN GUINEA PIGS DEPRIVED OF NERVE GROWTH FACTOR (NGF) IN UTERO. L. Sikich*, T.A. Woolsey & E.M. Johnson. Depts. of Anatomy & Neurobiology, & Pharmacology and McDonnell Center for Studies of Higher Brain Function, Washington Univ. Sch. Med., St. Louis, MO 63110.

In some rodents, somatotopically organized patterns corresponding precisely to the arrangement of the vibrissae are found at each level of the central trigeminal projections. In mice, the size of these representations is proportional to the peripheral innervation density (Lee & Woolsey, 1975). If groups of vibrissae are completely denervated during a defined critical period, their corresponding central representations are severely attenuated. We have studied animals in which all vibrissae were largely deafferented before birth.

Guinea pigs were exposed to maternal antibodies to NGF during the latter part of gestation. Newborn animals having high titers of the antibody were completely unresponsive to noxious stimuli. In affected animals, the infraorbital nerve was markedly smaller than that of normal animals and there were less than 20% the normal number of trigeminal ganglion neurons. Serial 50-µm sections tangential to the SmI cortex and transverse to the brainstem were stained for the mitochondrial enzyme, succinic dehydrogenase, in order to demonstrate the central vibrissae projection patterns. The representations in the spinal trigeminal nucleus and in the cortex were drawn using a camera lucida. Cross-sectional areas of the spinal trigeminal and cortical drawings of severely affected animals and normal animals were measured with an electronic tablet. The volume of the spinal trigeminal representation was calculated also. In spite of severe deficits in sensory function and neuron number, there were no significant differences in the sizes of the representations of the entire vibrissal field or individual vibrissal rows in either the spinal trigeminal nucleus or the cortex of severely affected animals as compared to normals. We conclude that in the guinea pig the total number of peripheral nerve fibers in prenatal life does not determine either the pattern or the size of the central representation of the vibrissae. (Supported by NIH grant NS 17763 and NS 18071, and the McKnight Foundation.)

(For 245.14 see 64.20)

245.15 **PRENATAL DEVELOPMENT OF THE NUCLEUS BASALIS /MEYNERT/ AND RELATED FIBER SYSTEMS IN MAN,** **I. Kostović, Section of Neuroanatomy, Department of Anatomy, Faculty of Medicine, University of Zagreb, 41001 Zagreb, Yugoslavia.**

Recent studies /Mesulam et al., JCN,214:170,1983/ suggest that large acetycholinesterase /AChE/ reactive neurons in the nucleus basalis /NB/ provide the single major source of cholinergic innervation for the cerebral cortex in the monkey. Evidence indicates a comparable cholinergic innervation of the human cortex: cell death which occurs in the NB in Alzheimer's disease results in a loss of cortical cholinergic markers. In order to provide new parameters for cholinergic innervation of the human fetal telencephalon we have analyzed the prenatal development of the histochemical reactivity in the NB. Brains from fetuses and premature infants ranging between 8 to 30 weeks of gestation /w./ were frozen cut and processed by thiocholine method for demonstration of AChE activity. The first sign of the histochemical differentiation of the basal telencephalon is the appearance of dark, AChE reactive "spot" situated between the anlage of the lenticular nucleus and the basal surface in a 9 weeks old fetus. The first AChE reactive bundle connects this area /nucleus basalis area-NBA/ with strongly reactive fiber system situated along the dorsal side of the optic tract. During the next stage /10,5 w./ there is significant increase in the size and reactivity of the NBA. At this stage we have seen AChE reactive bundle approaching /but not penetrating/ neocortical anlage through the external capsule. The supraoptic fiber system can be traced to the pregeniculate area and tegmentum. At 15 w. first AChE reactive perikarya appear and NBA become seggregated into several strongly reactive territories corresponding in position to the cell aggregations seen in Nissl preparations. At this stage NBA fibers from external capsule radiation enter "white" matter of the developing neocortex. In the next stage /18-22 w./ external capsule radiation penetrates transient subplate layer of the frontal, temporal and occipital lobe. In addition, strongly reactive fibers can be traced from NB to the subcortical structures: amygdaloid, caudate and mediodorsal thalamic nucleus. Between 22-28 w. there is significant increase in the number of large, strongly reactive neuronal perikarya in all divisions of NB: rostral, intermediate and caudal. In conclusion, NB develops earliest AChE activity in the telencephalon and sends fibers to the cortical anlage by the end of second trimester of gestation. These findings suggest that NB may be involved in early innervation of the cerebral cortex and developmental interaction with other growing afferents. **Supported by U.S.-Yugosl. Joint B. /I.K./**

245.16 IMMUNOCYTOCHEMICAL LOCALIZATION OF TUBULIN AND THE HIGH MOLECULAR WEIGHT MICROTUBULE-ASSOCIATED PROTEINS MAP1 AND MAP2 IN HIPPOCAMPAL NEURONS WHICH DEVELOP IN CULTURE. A. Caceres*, G. Banker, L. Binder*, and O. Steward. Department of Anatomy, Albany Medical College, Albany, New York 12208 and Departments of Neurosurgery and Biology, University of Virginia, Charlottesville, Virginia 22908.

Immunocytochemistry with monoclonal antibodies was used to study the distribution and development of β-tubulin, MAP1, and MAP2 in hippocampal cell cultures prepared from the brains of fetal rats. The binding specificity of each of the antibodies was determined by ELISA assays and cross reactivity to other proteins was assessed by staining immunoblots of extracts of whole hippocampus. In all cases the antibodies reacted only with their corresponding antigens.

After 2 weeks in culture, hippocampal neurons have elaborate processes which can be identified morphologically as dendrites and axons. Immunoreactivity for β-tubulin and MAP1 was found on both types of processes, as well as in the neuronal cell soma and in glial cells. In contrast MAP2 was only found in neurons, and within these cells was preferentially localized in dendritic processes. This pattern of labeling was the same for uninnervated, completely isolated cells in low density cultures as for cells in dense cultures, which receive heavy synaptic input. Hence this aspect of the molecular differentiation of the dendritic cytoskeleton occurs independently of innervation and despite the disruption of the normal temporal and spatial pattern of outgrowth which occurs in culture. It is likely that the three proteins analyzed in the present study are largely polymerized within the cytoskeletal network, since identical staining patterns were seen in Triton X-100 extracted cells (cytoskeletal preparations).

All three proteins were already present in cells at the time of plating. During the initial period of neuritic outgrowth (24-48 hrs after plating) all of the processes that emerged from the cells were equally immunoreactive for β-tubulin, MAP1, and MAP2. A gradual decrease in the immunostaining for MAP2 in the axonal processes was increasingly evident between 2 and 4 days after plating. By 7 days MAP2 staining had essentially disappeared from the axons, but remained present in the cell body and dendrites.

These results demonstrate that neurons in culture are capable of restricting MAP2 to dendrites, as they do in vivo. However, because MAP2 is initially present in both axons and dendrites, it seems unlikely that this protein plays a specific role in the early stages of dendritic development.

Supported by NIH grants NS17112 to G.B. and NS12333 to O.S.

245.17 IMMUNOHISTOCHEMICAL LOCALIZATION OF TUBULIN AND THE HIGH MOLECULAR WEIGHT MICROTUBULE-ASSOCIATED PROTEINS (MAP1 & MAP2) IN THE DEVELOPING CEREBELLUM OF THE RAT. A.Frankfurter,A.Caceres, L.I.Binder* and L.I.Rebhun*. Depts. of Neurological Surgery and Biology, University of Virginia, Charlottesville, VA 22908

We have demonstrated that the electrophoretic migration pattern of the high molecular weight brain microtubule-associated protein, MAP2,changes during development(Binder et.al.,this meeting).In the cerebellum,this change is apparent at approximately 18 days postnatally.Based on this observation,and a recent report suggesting a developmental role for the differential localization of the high molecular weight microtubule-associated proteins (HMW MAPs) and tubulin in dendritic differentiation,we have used monoclonal antibodies specific for MAP2, MAP1,the other HMW MAP,and beta-tubulin as probes to compare the distribution of these proteins during cerebellar development and adulthood.At all ages studied,5,10,15,20 and 30 days,tubulin and MAP1 immunoreactivities are associated with neuronal cell bodies,dendrites and axons, as well as with glia.In contrast,MAP2 staining is always markedly more intense in dendrites than in axons,and does not appear to be associated with glia.Moreover,at equal antibody concentrations, MAP2 staining is qualitatively more intense in axons of developing cerebellum.At all ages examined,the three antigens are colocalized within the Purkinje cell dendrite.The present study, thus,suggests that the major differences in distribution of these three cytoskeletal proteins are: 1)the apparent absence of MAP2 in glia,2)the qualitatively more intense staining of dendrites with MAP2 when compared to axons,and 3)the qualitatively more intense staining of axons with MAP2 during early development.The disparity between the results of the present study and those reported by others appears to be due to technical considerations. Immunoreactivity of tubulin,MAP1,and MAP2 is differentially affected by both fixation conditions and washing schedules employed prior to and during the peroxidase-anti-peroxidase procedure.

In the present study,we have demonstrated that tubulin and the HMW MAPs are colocalized at all stages of dendritic development. The electrophoretic pattern change found for MAP2 at 18 days, therefore,cannot be the result of an abrupt appearance and colocalization of de novo tubulin with HMW MAPs. Any hypothesis regarding the role of the HMW MAPs in dendritic differentiation must account for their colocalization with tubulin.The developmental significance of MAP2 heterogeneity remains to be determined.

Supported by NIH grant NS 17588 to A.F. and a grant from the Jeffress Memorial Trust to L.I.R.

245.18 ELECTROPHORETIC HETEROGENEITY OF MAP2 DURING DEVELOPMENT IN THE RAT CENTRAL NERVOUS SYSTEM. L. I. Binder*, A. Frankfurter, A. Caceras, M. R. Payne* and L. I. Rebhun* (SPON: J. Greenlee). Depts. of Biology and Neurosurgery, Univ. of Virginia, Charlottesville, Va. 22901, and Dept. of Anatomy, New York Medical College, Valhalla, N. Y. 10595.

MAP2 is a high molecular weight (ca. 300,000 M.W.) microtubule-associated protein, largely neuronal in distribution, which co-purifies with brain tubulin in vitro and binds helically along the length of the microtubule in the process. Using high resolution polyacrylamide gels, MAP2 isolated from adult brains is electrophoretically heterogeneous consisting of 2 closely migrating polypeptides: a slower migrating species, MAP2a and a faster migrating species, MAP2b. Both of these polypeptides bind to microtubules and behave in solution as monomers. Three monoclonal antibodies directed against different epitopes on MAP2 have been isolated and characterized. All of these react with both MAP2a & MAP2b.

During development of the cerebellum and cerebral cortex, the electrophoretic pattern of MAP2 changes. At 10 days after birth, nitrocellulose blots of SDS extracts from either whole cerebral cortices or cerebella display only 1 MAP2 band when probed with any of the MAP2 monoclonal antibodies. Moreover, taxol stabilized microtubules made from these areas of the brain at this time also show only 1 MAP2 band copurifying with tubulin. Coelectrophoresis experiments indicate that the MAP2 species present in the 10 day animal is MAP2b. MAP2a, the slower migrating form appears at day 18 in both cerebral cortex and the cerebellum.

In addition to these experiments, SDS extracts of spinal cords were also assayed for their MAP2 complement. Adult spinal cords contain 3 immunoreactive species of MAP2: MAP2a, MAP2b and MAP2c, a minor polypeptide species which migrates somewhat slower than MAP2a on SDS-polyacrylamide gels. At 10 days after birth, the spinal cord, unlike the cerebral cortex or the cerebellum already contains its adult configuration of MAP2a, b & c. This demonstrates that the change in MAP2 occurs differentially in distinct regions of the central nervous system. Finer characterization of regional MAP2 heterogeneity is currently under study. Also, experiments are currently underway to determine the cause of this developmental alteration and to ascertain its effects on the interaction of MAP2 with microtubules.

Supported by NIH grant NS 17588 to A.F. and also by a grant from the Jeffress Memorial Trust to L.I.R.

245.19 MONOCLONAL ANTIBODIES THAT RECOGNIZE GRANULE CELLS OF RAT DENTATE GYRUS. Joseph R. Moskal*, (Spon.: A. Bruner). Lab. Cell Biology, NIMH, Bethesda, MD 20205.

Monoclonal antibodies were generated against dentate gyri from the left hemispheres of 5 day old rat brains. Hybridomas were made by the fusion of spleen cells with NS-1 myeloma cells. The identification of antibodies that bind to the hippocampus was performed with unfixed, fresh-frozen, coronal sections of rat brain using immunofluorescent methodology. Hybridomas secreting antibodies of interest were further subcloned twice at limiting dilution to insure monoclonality. In this way 11 monoclonal antibodies were generated that gave two types of staining patterns. Antibodies of type 1 bound distinctly to 5 day old rat dentate granule cells showing cell body (intracellular punctate) and plasma membrane fluorescence. No staining of neuronal processes was observed and granule-like cells outside the hippocampus also were distinguishable. The dentate granule cell layer also was stained in adult rats. This included both granule cell bodies, as above, and their processes. Moreover extrahippocampal cell staining was markedly reduced. Both 5 day old and adult rat cerebellar staining was similar to that observed above.Type 2 antibodies gave a diffuse, nonspecific binding pattern to 5 day old rat hippocampus but appeared to bind to extracellular material located specifically among dentate granule cells in the adult. Again, cerebellar binding of these antibodies was similar to that described above. It was also possible, by radioimmunoassay, to detect specific antibody binding to washed crude membrane pellets prepared from adult dentate gyrus. Thus monoclonal antibodies have been generated that bind to dentate gyrus granule cells of the rat hippocampus. The binding patterns of these antibodies change during development,probably reflecting changes in the organization of the dentate gyrus. These results further suggest that the antibodies identify antigens on the cell surface.

245.20 SCANNING ELECTRON MICROSCOPIC (SEM) INVESTIGATION OF THE ORIGIN OF SUPRAEPENDYMAL NEURONS (SEN). J. D. Decker*, D. E. Scott. (SPON: J. Dexter). Department of Anatomy, University of Missouri-Columbia, School of Medicine, Columbia, Mo. 65212.

Supraependymal cell populations of the vertebrate cerebral ventricular system consist of at least two cell types. One cell type has been identified as a phagocytic cell, (e.g., the Kolmar cells) which presumably are of mesodermal origin and gain entrance into the cerebral ventricular system via the vasculature of the brain. A second cell type has been identified by SEM and transmission electron microscopy as a supraependymal neuron. This latter type of cell appears on the ventricular ependymal surface, concentrated in the regions of the circumventricular organs. The origin of the SEN cell line has not yet been determined.

Recently, in our laboratories SEM studies of the early development of the chick embryo provide evidence that the SEN have their origin from the cephalic transitory neural crest, (NC) and enter the ventricular system prior to the closure of the anterior neuropore. Age related differentiation of neurons at various stages of early embryonic development have been demonstrated. As NC cells migrate over the intraventricular ependymal wall of the forming third (thalamic) ventricle the transition from bipolar migrating cells to multipolar SEN has been recorded.

The discovery of a medial migration of NC cells over the ependymal surface of the embryonic chick cerebral ventricular system provides an excellent model for the analysis of the morphogenesis of NC cells and the correlates of putative neuroendocrine functions suggested for SEN.

245.PO TRACING THE INTRANEURAL TOPOGRAPHY OF THE MEDIAN NERVE THROUGH A COMPUTERIZED MOVIE. Julia K. Terzis, M.D., Ph.D. and Bradford L. Felker*. Microsurgical Research Center, Eastern Virginia Medical School, P. O. Box 1980, Norfolk, Va. 23501.

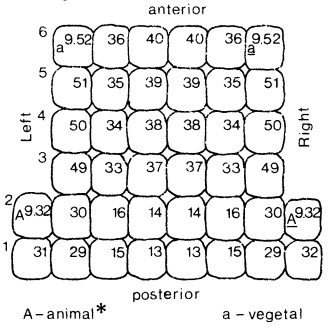
The intraneural topography of the median nerve was studied by examining one thousand five hundred (1,500) serial sections from a fresh cadaver median nerve. Each section was cut at sixty micron thickness. Sections were examined sequentially at 200 micron intervals from the middle arm level to the fingertips. The nerve was frozen and sections were cut using a freezing microtome (American Optical Histostat Microtome). Each section was projected to a semitransparent board and tracings were made. The exact perimeter of the outer epineurial sheath of the nerve trunk was recorded along with the constituent fascicular bundles by tracing their perineurial sheath. The intraneural fascicular organization was thus depicted at each level and the anatomical positions and exit points of the sensory and motor components accurately recorded. Each tracing was then encoded into a Crommemco System Three microcomputer using a Talos Digitizing Tablet computer. Each section was then serially recalled, displayed on a graphics screen and filmed using time lapse photography. The resulting movie illustrates for the first time the complex arrangements of the intraneural bundles in their journey to the periphery. The digitally computerized film traces motor and sensory fascicles in their natural anatomical positions as they maneuver their way within the epineurial sheath, split, rejoin, migrate and branch upon their exit from the main trunk for their respective muscles and cutaneous receptive fields.

It is hoped that data such as this study provides will greatly increase our understanding of the sensory-motor distribution and the intraneural organization of peripheral nerve trunks which is vital for functional restoration procedures following peripheral nerve injury and repair.

CELL LINEAGE AND DIFFERENTIATION I

246.1 CELL MAPS AND LINEAGE IN THE DEVELOPING NERVOUS SYSTEM OF THE ASCIDIAN TADPOLE LARVA. D. Nicol and I. A. Meinertzhagen. Dept. of Biology, Dalhousie Univ., Halifax, N.S., Canada B3H 4J1.

Larvae of tunicates (Urochordata: Ascidiacea) have a dorsal tubular nervous system widely touted as a signal feature of the group's chordate ancestry. In particular, the tunicate neural tube arises during development in the familiar vertebrate pattern, as an ectodermal neural plate which rolls up longitudinally. In other ways the embryo falls outside chordate orthodoxy. Its mode of development is mosaic and its cleavage apparently eutelic, yielding constant numbers of cells (i.e. neurones), properties more in common with various invertebrate groups. We have examined the early development of the embryo of Ciona intestinalis, from SEM and serial-section reconstructions of staged embryos, to investigate the morphology and lineage of the neurones of the larval nervous system, in an attempt to define some of the factors which generate the fundamental architectural features of the vertebrate nervous system.



The lineage of the embryo in Ciona is known from Conklin (1905) up until the 218-cell stage. At this time (7 hr. at 20°C) the neural plate contains 40 cells. These are arrayed in four rows of six and two rows of eight cells, as shown. Rows 1 and 2 divide first followed by rows 3-6, which divide in a medial to lateral and posterior to anterior sequence. By the time (9 hr., 20°C) row 3 has divided, taking the number of neural plate cells to 62, rows 1 and 2 have already commenced neurulation. At the end of neurulation (12 hr., 20°C), some of the daughters of rows 1 and 2 have divided again, taking the total number of row 1 and 2-progeny to about 56. These cells contribute the ependymal lining of the neural tube which comes to lie in the tail of the mature larva. The ependymal cells are arranged so that four comprise the neural tube cross-section, two laterally and one each dorsally and ventrally. At this stage there are 48 anterior cells constituting the brain region. These are the daughter cells of rows 3-6 and they go through at least one further division to complete their lineage.

Supported by grant A-0065 from NSERC.

*lineage nomenclature after Conklin (1905).

246.2 DEVELOPMENT OF PHOTORECEPTORS IN ZEBRAFISH. L. W. Nawrocki. Institute of Molecular Biology, Univ. of Oregon, Eugene, Oregon 97403.

Cone photoreceptor cells form a regular mosaic in the retina of the zebrafish, Brachydanio rerio (as previously observed for many other teleosts). The mosaic consists of alternating columns arranged along the dorso-ventral axis. One column is made of alternating single cones with absorption maxima at 415nm and 480 nm. The other column (two cells wide) is made of alternating rows of pairs of double cones with absorption maxima at 480nm and 550nm. (Absorption maxima of adult and young cells were measured in collaboration with M. Kaplan, R. Bremiller, and G. Streisinger.) Rod outer segments (503nm abs. max.) are located sclerally above the cones. The development of these receptors was investigated by light microscopy and their birthdates were established by tritiated thymidine labelling.

All cells in the presumptive neural retina are fusiform and histologically homogenous from day 0.5 (after fertilization) until day 2.4. Mitotic division giving rise to the neurons of the central retina occur at the ventricular surface. At day 2.4, the outer nuclear layer is formed, and all the cone cells in the central retina have completed their last mitotic division. These immature cones maintain a single layer of nuclei. Outer segments and synaptic terminals appear at day 3.0 in the central retina; on day 4, the nuclei of these cones have formed two layer in the outer nuclear layer, and on day 5, the first short single cones have moved to their mature position with their outer segments and their ellipsoids separated by the external limiting membrane. All three cone visual pigments have been identified in day 7 photoreceptor outer segments by microspectrophotometry. On day 9, the regular adult mosaic is present in cross section.

The first outer segments seen in the entire retina are those of precocious rods located close to the ventral retinal margin. Their nuclei lie close to the outer plexiform layer, and their outer segments are of mature diameter. Rod visual pigment has been measured with microspectrophotometry as early as day 6. On day 8, rods are found close to both the ventral and the dorsal margin. By day 12, rod outer segments are present in the central retina, but these rods had later birthdays than the cones in the same region.

The initial development of the zebrafish retina is thus quite different in the central than the marginal area. Central cones appear and develop into their mature forms prior to the appearance of rods (as has been established by T. Branchek). In contrast, at the ventral margin rods appear precociously, and exhibit mature outer segments, considerably before the appearance of cones.

246.3 SIMULTANEOUS EXPRESSION OF NEURONAL AND GLIAL PROPERTIES BY CHICK SENSORY AND AUTONOMIC GANGLION CELLS DURING DEVELOPMENT, H. Rohrer and I. Sommer* (SPON: Y.-A. Barde). Max-Planck-Institute for Psychiatry, D-8033 Martinsried, FRG and *Institute for Neurobiology, University of Heidelberg, D-6900 Heidelberg, FRG.

There is evidence to indicate that cells in cholinergic ciliary and sensory ganglia are able to acquire characteristics of adrenergic neurons when transplanted into trunk neural axis of stage 14 chick embryos. Recently it has been demonstrated that the adrenergic neurons do not originate from neurons but from non-neuronal cells present in the ganglion at the time of implantation (Ayer-Le Lievre and Le Douarin, Devel. Biol. 94: 291-310 1982). In the present study we have examined whether the non-neuronal cells of sensory or autonomic ganglia already express properties of adrenergic neurons before transplantation. Non-neuronal cells from dissociated chick dorsal root (DRG), sympathetic (SY) or ciliary (CIL) ganglia, grown in cell culture for 1 day, exhibit high affinity uptake for norepinephrine (NE) and/or specific receptors for nerve growth factor (NGF). Cells with NE uptake or NGF receptors were identified by autoradiographic methods, and such cells reacted with neither tetanus toxin nor with antibodies to neurofilament proteins or fibronectin. They were, however, positive for 04 antigen, which is recognized by a monoclonal antibody. This antigen is present on Schwann cells and oligodendrocytes (Schachner et al., Devel. Biol. 83: 328-338 1981).

At all ages studied between embryonic day six (E6) and embryonic day sixteen (E16), 70 to 80% of the non-neuronal cells were positive for 04 antigen in all ganglia analysed. 04 negative non-neuronal cells could be identified as fibroblast-like cells by staining with antibodies to fibronectin. The proportion of 04 positive cells with NGF receptors and NE uptake decreased during development. The percentage of 04 positive cells which have NGF receptors decreased from about 95% at E6 to between 10% (E16 SY) and 35% (E14 CIL and E16 DRG). The proportion of 04 positive cells with NE uptake decreased from 30 - 50% at E6 to between 3% (E16 SY and E16 DRG) and 15% (E14 CIL). We suggest that those cells exhibiting biochemical properties of both differentiated glial cells and neurons are precursor cells which have the potential to develop into either glial cells or neurons.

246.4 ENTERIC NEUROGENESIS BY NEURAL CREST-DERIVED BRANCHIAL ARCH MESENCHYMAL CELLS OF BIRDS. G. Ciment and J.A. Weston*. Dept. Biology University of Oregon, Eugene, OR 97403.

In earlier work (Devel.Biol.93:355,1982), we reported the establishment of a hybridoma cell line secreting a monoclonal antibody (E/C8) which recognizes an avian-specific epitope found in some cultured neural crest cells, both central and peripheral neurons in vivo, and in a limited number of non-neuronal tissues, including mesenchymal cells of the posterior (3rd and 4th) branchial arches. These latter tissues are transient embryonic structures which serve as the lateral and ventral walls of the primitive pharynx of vertebrates and are contiguous at the caudal end with the newly developing gut.

We report here that the antigen(s) recognized by the E/C8-monoclonal antibody in mesenchymal cells of the branchial arches and in peripheral neurons are the same. Moreover, the E/C8-positive mesenchymal cells of the arches can develop into morphologically-recognizable neurons either spontaneously in culture, or in tissue cocultures with aneural portions of the gut (where these branchial arch cells form apparently normal enteric ganglia). In contrast, these cells do not develop into melanocytes--another derivative of the neural crest--under several permissive conditions.

These data indicate that the mesenchymal cells of the posterior branchial arches are a population of partially-restricted neural crest-derived cells and may serve as precursors for neurons of the enteric nervous system.

Supported by NIH grant DE-04316 and NSF grant PCM-8218899.

246.5 MONOCLONAL ANTIBODIES AGAINST THE CHICK EMBRYO SPINAL CORD. H. Tanaka* and K. Obata Dept. of Pharmacology, Gunma Univ. Sch. of Med., Maebashi 371 Japan.

Molecular differences among neuronal cell surfaces will develop in the early stages of the embryo and play a role in cell-cell interactions leading formation of neuronal network. These differences will give specific identification markers of cell types in vivo and in vitro. Investigation on such molecules becomes possible with the development of the monoclonal antibody technique.

Mice were immunized by injecting crude membrane preparations of 6- and 13-day-old chick embryo spinal cords. Standard technique was used for fusion with X63-Ag8-653 cells. Hybridoma lines were screened by indirect immunofluorescence on 5 um thick paraffin sections of the chick embryo trunk. Chick embryos had been fixed and dehydrated in acetone at -80°C.

Of 700 cell lines screened, approximately 55% gave positive staining on spinal cord. Of these, 42% stained only the neuronal tissues, 13% stained neuronal and several non-neuronal tissues, and 45% stained all cells nonspecifically. Twenty independent lines were established. We described here 4 examples studied in detail. Since some staining pattern differed among trunk levels reflecting rostrocaudal gradient in the developmental stages, thoracic level was mainly investigated.

Antibody CESC 1 stained nerve fibers of spinal cord and dorsal root ganglion (DRG) cells. The staining appeared first on spinal cord cells at external mantle layer at stages 20-22 (Hamburger and Hamilton) and then DRG cells. In the later stages in spinal cord, the staining on somatic and visceral motoneurons was remarkable. Antibody CESC 2 stained dorsal funiculus but not cells in spinal cord. Outside the spinal cord, the staining was restricted on the surfaces of DRG and sympathetic ganglion cells and their fibers. However, the staining was not observed on neural crest and appeared after differentiation of ganglion cells at stages 24-25. Antibody CESC 3 stained the cell surfaces of both spinal cord and notochord from stage 16. Weak staining was also observed on mesonephros and somite and later on muscle cell surface. Staining on notochord disappeared at later stages. Antibody CESC 4 first stained notochord from stages 24-25 and then the spinal cord.

Monoclonal antibodies against embryonic nerve tissue, thus, revealed neuronal heterogeneity and development of antigenic determinants and their coexistence in such embryonic tissues as notochord and mesonephros.

246.6 NEURONAL AND GLIAL DIFFERENTIATION IN THE CERVICAL SYMPATHETIC GANGLIA OF THE RAT. K. Droms* and N. Sueoka* (SPON: B. L. McNaughton). Dept. of MCD Biol., University of Colorado, Boulder, CO 80309.

The neural crest origin of the neurons and glia of the sympathetic ganglia has been well documented. However, the developmental stage at which crest cells become committed to the neuronal and glial lineages has not been established. We have been examining this commitment by correlating the appearance of biochemical markers with morphological differentiation of neuronal and glial cells in the cervical sympathetic ganglia of the rat. Neuronal and glial cells first become morphologically distinguishable at embryonic day 14 (E14; EO = conception, E22 = birth). The S100 antigen, however, is not expressed in glial cells in the ganglia until after birth. The uptake of ^{3}H-GABA by glial cells is expressed much earlier in development (E15). At this stage only morphologically distinguishable glial cells take up GABA although many morphologically indistinguishable cells persist within the ganglion. Therefore, our observation is that morphology is the earliest detectable marker of glial differentiation so far found in this system.

We have also been studying the development of sympathetic ganglia in tissue culture. When E13 ganglia are cultured in our standard medium (DME, 12.5% horse serum, 2.5% fetal calf serum) supplemented with NGF no morphological differentiation of neuronal or glial cells can be detected. However, E14 ganglia plated in the same condition do exhibit neuronal differentiation, as evidenced by the extension of long cellular processes from the tissue explant. Hematoxylin and eosin stained sections of E14 ganglia after four days in culture display distinct morphological differentiation of neuronal and glial cells. Neuronal differentiation in E13 ganglia, as indicated by the extension of long processes from the tissue explant, does occur if the ganglia are cultured in medium without fetal calf serum (FCS). Sections of these cultured ganglia do exhibit two distinct cellular morphologies; one "neuronal-like" and one "glial-like," but the morphological differentiation of these cells is not as distinct as in ganglia cultured from later developmental stages. Ganglia from E14 and later developmental stages cultured in standard medium are indistinguishable from ganglia cultured in medium without FCS.

We are currently investigating the several possible interpretations of these phenomena. One exciting possibility is that E13 represents the stage at which commitment to the neuronal and glial lineages is in progress. We will test this idea by studying the relative dynamics of the neuronal and glial population in vivo and in vitro and by attempting to affect the choice of lineage by cultured ganglionic precursor cells.

246.7 DISTRIBUTION OF GLIAL CELL PROCESSES IN RETINA TRANSPLANTED TO THE RAT BRAIN. S. C. McLoon and H. J. Karten. Dept. of Anatomy, Medical University of South Carolina, Charleston, SC 29425 and Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11790.

Previous studies have shown that fetal rat retina will survive and continue to differentiate when transplanted adjacent to the superior colliculus of newborn hosts. The neuronal elements of these retinal transplants appear identical to their counterparts in normal retina for all characteristics studied so far. In the present study we have used immunohistochemical techniques to characterize the glial elements in the retinal transplants and compared these to the glial elements of retina in situ. The Müller cell processes of normal retina run from outer to inner limiting membrane as can be demonstrated by the Müller cell specific antibody G3 (developed by C. Barnstable), while an antibody to glial fibrillary acidic protein (GFA) only stains processes in the optic fiber layer. The GFA positive cells appear to be astrocytes that migrate into the retina along the optic nerve during the first post-natal week. At birth the GFA reactivity was confined to the optic nerve head and optic fiber layer immediately surrounding the nerve. During the next five days, the GFA reactivity expanded out from the optic nerve concentrically. By injecting colchicine into the eye or by injuring the retina, Müller cells develop a GFA antigenicity. In the retinal transplants, however, the staining pattern of both antibodies was the same. Glial fibers which appeared to be both GFA and G3 positive ran from the outer limiting membrane at the center of the photoreceptor rosettes through the ganglion cell layer. It is not clear at this point if the glial cells in the retinal transplants are astrocytes which migrated in from the host brain and which underwent a gene modification in the retinal environment to express Muller cell specific antigenicity or are Müller cells expressing an injury-like reactivity to the GFA antibody. It is interesting that in the disturbed environment of the transplants the glial processes were still capable of orienting radially through all the cell layers.

(Supported by grants EY04627 to SCM and EY04796 to HJK from the NIH)

246.8 TEMPORAL SEQUENCE OF GRANULE CELL MIGRATION IN VITRO. E. Trenkner and N. Segil*. Dept. of Pharmacology, New York Univ. Medical Center, New York, NY 10016.

Questions of intrinsic vs. extrinsic control of cellular behavior have been central to the analysis of neuronal development. The reaggregating cerebellar culture system of Trenkner and Sidman (1) has been useful as an in vitro model for the study of granule cell migration during cerebellar development. In the present study we have confirmed the identity of granule cells in these cultures and described the temporal sequence of granule cell development and migration.

At postnatal day 3 (P3), the majority of cells dividing in the cerebellum are external granule cells, a subpopulation of which are destined to begin migrating within 24 hrs. (2). Therefore, we injected ^{3}H-thymidine into P3 animals and at various times thereafter prepared cerebellar cultures in order to determine whether this labeled, migrating population of cells appeared as migrating cells in cultures. 3 days after culture of P4 animals (injected 24 hrs earlier) 30%-40% of the migrating cells were labeled. This % was much lower in cultures prepared 96 hrs (P7) after ^{3}H-thymidine injection, indicating that there might be predetermined length of time during which these cells can migrate. To test this hypothesis, P3 cerebellar cells were labeled and then cultured for various times up to 7 days. The largest % of labeled cells on cables was found after 3 days in vitro. Subsequently, the % of labeled cells on cables declined to near 0, most probably as a result of migration into reaggregrates (1). Unlabeled cells continued to migrate on cables indicating that the cultures were still capable of supporting migration.

Reaggregates were studied in 1 sections prepared for autoradiography. Increased numbers of labled cells appeared in reaggregates with increased time in culture. This increase correlated with the increase in number of labeled cells in the internal granule cell layer in vivo. Also, the number of labeled cells observed in reaggregates was dependent on length of time after final mitosis. Cultures from animals labeled at P3 and cultured at P4 contained considerably fewer labeled cells in reaggregates than those cultured at P7.

This study confirms the electon microscopic evidence that the cells migrating on cables are granule cells and suggests that there may be a predetermined length of time for granule cells to migrate.

(1) Trenkner, E. and Sidman, R.L. (1977) J. Cell Biol. 75:915.
(2) Fugita et al. (1966) J. Comp. Neur. 128:191.
Supported by NIH grant NS-16071 to E.T.

246.9 VENTRAL NEURAL TUBE AND NOTOCHORD OF RAT EMBRYOS CONTAIN AROMATIC L-AMINO ACID DECARBOXYLASE. G. Teitelman, C.B. Jaeger, V.R. Albert, T.H. Joh and D.J. Reis. Laboratory of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

In vertebrate embryos, the catecholamine (CA) neurons of brain and periphery arise from undifferentiated precursors in the ventral neural tube and neural crest to migrate to their final location where they first express tyrosine hydroxylase (TH). We sought to determine by immunocytochemistry and autoradiography whether the next enzyme of the CA pathway, Aromatic L-Amino Acid Decarboxylase (AADC) is expressed by CA precursors before or, as TH, after reaching their final site and by combining immunocytochemistry and radioautography of tritiated thymidine (^{3}H-thy) whether AADC is present in proliferating cells.

Rat embryos and adult tissue were fixed in 4% paraformaldehyde, sectioned and stained for AADC according to the PAP technique. For simultaneous radioautographic demonstration of labeling of AADC by ^{3}H-thy, pregnant rats were injected at the 14th day of development (E 14) with 5 uCi/g of the isotope and killed two hours later. After processing the embryonic tissue for immunocytochemical localization of AADC, the sections were coated with Kodak NTS$_2$ emulsion, exposed to two weeks in the dark, developed, fixed and mounted.

AADC was first observed on E 12 in all cells of the notochord along its entire length and in neuroepithelial cells of the ventral neural tube from the mesencephalic to the lumbar region. At E 14 the cells of the sacral basal plate of the neural tube also contained AADC. At this stage, some cells of the notochord and neural tube containing AADC were also labeled with tritiated thymidine. At E 16 the cells of the notochord lacked AADC immunoreactivity and at E 21 the enzyme was no longer detected in the ventricular layer of the developing brain. At this stage and in the adult stage, however, a few cells containing AADC were found scattered in the spinal cord. AADC was not detected in migrating neural crest cells.

This study indicates that in rat embryo: (1) AADC is expressed by cells of the ventral neural tube and notochord from E 12 until late gestation; (2) the onset of expression of AADC occurs prior to the last cell division; and, (3) at least in brain, the precursors of CA neurons express AADC while still in their site of origin, and TH after arriving at their final location.

246.10 GLUCOCORTICOID-INDUCED DELAY OF THE RESPONSE OF CULTURED ADRENAL CHROMAFFIN CELLS TO NERVE GROWTH FACTOR. L.E. Lillien and P. Claude. Wisconsin Regional Primate Research Center and Neurosciences Training Program, Univ. of Wisconsin-Madison, WI 53706.

Adrenal chromaffin cells from young rats form neurites and exhibit ultrastructural features that are characteristic of neurons when they are grown in vitro in the presence of Nerve Growth Factor (NGF) (Unsicker et al., 1978; Doupe, 1982). Glucocorticoid hormones have been reported to inhibit this response to NGF (Unsicker, 1978). We have followed the effects of dexamethasone (dex) on the NGF-induced changes in chromaffin cells over several weeks and find that dex appears to delay rather than completely inhibit the NGF response. Chromaffin cells grown in the absence of NGF (control cultures) are small (10-15 um in diameter), flat and polygonal and very few have neurites. No large, round cell bodies typical of neurons are present in control cultures. In the presence of NGF (100 ng/ml 7S NGF), the proportion of neurite-bearing chromaffin cells increases from approximately 15% after 1 week, to 40% at 2 weeks, to over 70% by 4 weeks in culture; the proportion of large spherical neuron-like cells (20-30 um in diameter) increases from 0% at 1 week and 1-2% at 2 weeks to approximately 80% by 4 weeks. In the presence of NGF plus 10^{-5}M dex (Decadron, Merck), the proportion of neurite-bearing cells at 1,2 and 4 weeks is approximately 3%, 10% and 20-50%, respectively; large spherical cells are not apparent at 1 or 2 weeks, and constitute 10% of the chromaffin cells present at 4 weeks. If chromaffin cells are grown for 1 week in the presence of dex alone, followed by the removal of dex and addition of NGF (100 ng/ml), the initiation of neuritic outgrowth is apparent about 4 days after the switch; when sister cultures are grown for 1 week under control conditions (no NGF or dex) then switched to NGF, process outgrowth is apparent by 2 days after the switch. These results also suggest a delaying effect of dexamethasone on the neuritic response of chromaffin cells to NGF. Possible mechanisms for this delay, specifically, a direct effect of dex on the binding or processing of NGF by chromaffin cells will be investigated, as well as a possible mitogenic effect of dex on adrenal chromaffin cells. Preliminary evidence from cultures labeled in vitro with tritiated thymidine suggests that while chromaffin cells divide in vitro under all conditions, the proportion of labeled cells in cultures grown in NGF plus dex is greater than in cultures grown in NGF alone.

Supported by NIH grant RR00167 to the Primate Research Center and by a Research Grant from the Muscular Dystrophy Assoc.)

247.1 CHANGES IN NEURONAL NUMBER IN THE DEVELOPING HAMSTER VISUAL SYSTEM FOLLOWING MANIPULATIONS OF TARGET AVAILABILITY. John Kirn*, Judy I. Raabe* and Barbara L. Finlay. Dept. of Psychology, Cornell University, Ithaca, N.Y. 14853.

The control of cell number through naturally occurring cell death in complex, interconnected systems is as yet poorly understood. Here two manipulations in the visual system designed to remove one retinal target and observe changes in both retina and other targets are examined, with regard to the relative contributions of afference and efference in the control of cell number.

In prior research designed to test the effect of increased afference (Wikler and Finlay, 1982) no change in cell death in residual tectum was observed following caudal unilateral tectal lesions in neonatal hamsters (48%-79% reduction in surface area). Our experiment examined cell loss in the retinal ganglion cell layer of these animals. Cell counts in the ganglion cell layer in horizontal sections through the retina were made on postnatal days 4-8, the period of maximal cell death in hamster visual system. The number of degenerating cells, recognized by their pyknotic appearance in light microscopy, were expressed as a fraction of normal cells. No difference in cell degeneration rates between eyes contralateral and ipsilateral to the lesion were observed. However, cell loss in peripheral retina of both eyes was higher than in normal hamsters on postnatal days 6-8. The caudal tectum does not receive bilateral projections in the adult. However, caudal unilateral tectal lesions performed neonatally apparently produce a bilateral reduction in retinal target availability.

Visual cortex removal in neonatal hamsters results in degeneration of the dorsal lateral geniculate nucleus and a potential excess of retinal innervation to other retinal targets such as the superficial layers of the superior colliculus. This manipulation also denervates posterior thalamic nuclei where visual cortex and superior colliculus both project, potentially increasing thalamic targets for superior colliculus efferents. The right visual cortex was removed on the day of birth in hamsters. The geniculate was reduced to 63% of its normal surface area by postnatal day 4 with progressively more degeneration seen on later days. Assessments of cell degeneration rates were made in superficial and intermediate layers of the superior colliculus on postnatal days 4-8. Rates of cell loss in superficial layers contralateral and ipsilateral to the cortical lesion were identical, suggesting no effect of potential excess afference ipsilateral to cortical removal on cell survival. In the intermediate and deep layers however, 6 out of 8 animals showed decreased cell loss ipsilateral to cortical removal, suggesting that the target area was increased for intermediate layer efferents.

These results suggest that target availability and not afferent supply is the primary factor controlling cell number in complex interconnecting neuronal populations.

Supported by NSF grant BNS 79 14941.

247.2 EFFECT OF ISCHEMIA-LIKE CONDITIONS ON NEURONAL SURVIVAL IN CELL CULTURE W.J.Goldberg*, R.M. Kadingo*, and J.N. Barrett. Dept. of Physiology and Biophysics, University of Miami Sch. of Med., Miami, FL 33101

One of the major problems in the study of ischemia in vivo is the inability to dissociate the direct effects of oxygen and glucose deprivation on neurons from the secondary effects which result from vascular changes. We present here an in vitro system for assessing the ability of neurons to withstand and recover from the combined deprivation of oxygen and glucose. Dissociated cultures of basal ganglia cells (striatum and substantia nigra) from 14-16 day gestation rat embryos were maintained in nutrient medium supplimented with a survival factor isolated from horse serum (Kaufman and Barrett, Science in press). When cultures were deprived of both glucose and oxygen in the medium (PO_2 below detectable level using Pt electrode) for a period of three hours more than 90% of the neurons became swollen and had irregular nuclei. Within 24 hours the cells were replaced by debris. The sensitivity of neurons to this ischemic insult was found to be highly dependent upon the age of the culture. Cultures less than three weeks old were much less sensitive and at less than two weeks of age were not significantly damaged by this three hour deprivation of glucose and oxygen. We find that the combined deprivation of glucose and oxygen was much more damaging to neurons in culture than was anoxia alone since oxygen deprivation for three hours produced little or no damage. It has recently been reported that 14 hours of exposure to anoxic conditions does result in the death of neurons in culture (Rothman, Science, 220:536).

Both young and old neuronal cultures were not affected by many of the microenvironmental alterations which occur during ischemia in vivo. High ammonia (up to 5 mM), high lactic acid (up to 20 mM) and low pH (to 6.5) did not severely damage the cells provided that glucose and oxygen were present.

(Supported by NIH grants #NS-07044 and #NS-12207 and by the National Parkinson Foundation.)

247.3 REGULATION OF NEURON NUMBERS IN XENOPUS: EFFECTS OF HORMONAL MANIPULATION ALTERING SIZE AT METAMORPHOSIS. D. G. SPERRY* and P. GROBSTEIN (SPON: J. Siegel). Sch. of Life and Health Sci., Univ. of Delaware, Newark, DE 19711 and Com. on Neurobio., Univ. of Chicago, Chicago, IL 60637.

The developmental mechanisms that operate to ensure a functional relationship between nervous system organization and body size are subjects of continuing interest. In frogs, the numbers of neurons with peripheral processes may be determined by signals relating peripheral size to the amount of naturally occurring neuronal death during metamorphosis. Neither the exact timing of such hypothetical signals nor the particular aspects of peripheral size they might relate are known.

To provide new information on neuron number regulation we studied frogs where metamorphosis was delayed and peripheral size at its completion was greatly increased by interfering with the hormonal balance necessary for metamorphosis at the normal rate. X. laevis tadpoles living in rearing medium containing the anti-thyroid agent propylthiouracil cease development at a late larval, hindlimb bud stage prior to the onset of motoneuron death. Animals remain viable, continue growing, and, when returned to standard rearing medium, complete normal metamorphosis but at an increased size.

Counts of lumbar lateral motor column motoneurons were made on four normal postmetamorphic frogs and four experimental post-metamorphic frogs. The experimental frogs completed metamorphosis six months later than their untreated siblings and had bodies of normal proportions but four to five times larger. Despite these size differences, motoneuron numbers were the same in both groups. We also counted muscle fibers in two hindlimb muscles. Surprisingly, muscle fiber numbers were not greater but actually diminished in the experimental animals. Our results indicate that the hypothetical signal regulating motoneuron numbers is not related to gross peripheral size during late metamorphic stages. The results leave open the possibility that the signal is related to muscle fiber number, although we did not observe a decrease in motoneuron number proportional to the decrease in muscle fiber number in the experimental animals.

Counts of dorsal root ganglia cells are in progress. Preliminary findings suggest an increase in neuron number in the thoracic ganglia porportional to the increase in body surface area of the experimental animals but no increase in those ganglia associated with the hindlimb. A difference between sensory and motor systems and between thoracic and lumbar body regions would be of interest in further analyses of possible peripheral regulation of neuron numbers.

Supported by Biomed RR07016-08, UDRF, and NSF BNS 7914122

247.4 DECREASED MOTOR NEURON SURVIVAL IN THE DEVELOPING MUSCULAR DYSTROPHIC CHICK. P.A. Stewart and M.P. Rathbone. Department of Anatomy, University of Toronto, Toronto, Ontario, and Department or Clinical Neurological Sciences, University of Western Ontario, London, Ontario CANADA.

Inherited muscular dystrophy in the chicken is characterised by a variety of muscular and neural abnormalities. In view of the early expression of dystrophy in the muscles one might predict a deficit in the motor neuron population. However, studies on the New Hampshire strain of dystrophic birds maintained in Davis, CA, have shown that the population of the lateral motor column (LMC) is normal. Since the expression of the dystrophic gene in birds is modofied by background genes, we questioned whether dystrophic chicks that have been outbred to White Leghorn, and maintained in Storrs, CT, might have abnormalities in their motor neuron pools.

We estimated total numbers of motor neurons in the brachial LMC in normal and dystrophic chicks at 6, 11 and 17 days of development and at 3 days post-hatching. Since motor neuron populations vary among White Leghorn sub-strains, we examined both of the dystrophic lines maintained at Storrs and, as controls, used the inbred normal birds supplied by the same facility and also locally obtained White Leghorns. We found that the motor neuron population was the same in all 4 lines at 6 days in ovo but by 11 days in ovo and thereafter both dystrophic lines contained about 80% of the normal number. Both normal lines had approximately the same population.

To determine whether the expression of dystrophy in the target muscles might affect the survival of motor neurons we transplanted the presumptive brachial region of the spinal cord between normal and dystrophic embryos at 2 days in ovo and evaluated the LMC population at 17 days in ovo in the resulting chimeras. We found that both normal and dystrophic spinal cords transplanted into dystrophic hosts had significantly fewer motor neurons than normal and dystrophic spinal cords transplanted into normal hosts. In fact, dystrophic cords transplanted into normal hosts had slightly more neurons than in unoperated dystrophic birds. Therefore we suggest that an interaction with normal muscle fibers was able to rescue a percentage of dystrophic motor neurons that would have died in their native environment.

We conclude that in the Storrs strain of dystrophic birds the observed deficit in the brachial LMC is secondary to some influence of the dystrophic target muscles.

Supported by the Medical Research Council of Canada and by the Muscular Dystrophy Association of Canada.

247.6 TARGET TISSUE- AND SUBSTRATUM-DEPENDENT SURVIVAL OF LATERAL MOTOR COLUMN NEURONS IN LONG-TERM SPINAL CORD EXPLANT CULTURES.
E.D. Pollack, V.L. Liebig* and W.L. Muhlach* (SPON: K.R. Swiatek). Inst. Study of Develop. Disabil. and Dept. Biol. Sci., University of Illinois at Chicago, Chicago, IL 60608.

Both target tissue and the attachment substratum are critical for the in vitro survival of lateral motor column (LMC) neurons of tadpole spinal cord explants grown in defined medium (Pollack, Neurosci Abstr.,'82). We previously demonstrated that the number of neurons in the LMC after 21 days of culture was optimal in the presence of mesenchymal limb bud tissue and polylysine substratum. Longer term cultures have now been analyzed in order to assess the specific organotypic organization of cord explants with respect to potential maturational changes in the LMC resulting from continual peripheral target influences. Lumbosacral spinal cord and mesenchymal limb tissue were derived from young (stage V) Rana pipiens tadpoles and explanted on either collagen or poly-DL-lysine substrata.

Cord explants grown on collagen without target tissue could not be maintained beyond 4-5 weeks. By this time they exhibited no neuritic outgrowth and a generally disorganized cytoarchitecture lacking clearly delineated LMCs. The presence of limb bud tissue promoted explant survival with enhanced neuritic outgrowth for periods that can exceed two months. An organized LMC persisted with neurons that are apparently more mature than that seen after only 3 weeks of culture. Cords grown on polylysine without target tissue also survived for prolonged periods with typically well-formed LMCs. However, the LMC had a reduced neuronal population and contained larger numbers of necrotic cells than when target was present with cord explants on polylysine.

Of developmental significance is the finding that cord cultures with target tissue generally had an LMC present or prominent on one side only. This is further reflected in the fact that long-term cultures showed degeneration of what earlier had been healthy neurites in areas not spatially related to the target tissue. This more specific effect of the target was not seen in shorter term cultures. Group comparisons suggest that the target tissue offers a survival effect beyond an apparent stabilizing influence imparted by the polylysine substratum. Several cord explants were cultured with limb conditioned medium for 4 weeks during which time they extended target-dependent neurites and exhibited viable LMCs on both sides of the cord.

The results thus far indicate that the normal mesenchymal limb target for LMC neurons promotes selective long-term survival of those neurons in vitro while the nature of the substratum may provide a more generalized cellular stability that contributes to overall survival. (Supported in part by NIH grant NS 13814).

247.7 THE EARLY POSTNATAL RESTRICTION OF THE IPSILATERAL RETINOCOLLICULAR PROJECTION IS DUE TO CELL DEATH RATHER THAN COLLATERAL ELIMINATION.
Dennis D.M. O'Leary, James W. Fawcett,* and W.M. Cowan, The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

Early in the postnatal life of rodents that have been studied there is a substantial ipsilateral retinocollicular projection. In newborn albino rats this projection extends throughout the colliculus, and arises from ganglion cells that are scattered throughout the ipsilateral retina. After the first few days postnatally this early extensive projection becomes progressively restricted, and by postnatal day 10 it is largely limited, as it is in adult rats, to the rostromedial part of the colliculus (Land and Lund, Science, 205:698, 1979).

To determine whether this restriction is due to collateral elimination or to the death of the ganglion cells from which the extensive projection arises we have used the retrogradely transported fluorescent tracers Fast Blue (FB) and Diaminido Yellow-dihydrochloride (DY), which label the cytoplasm and nucleus respectively, as long-term neuronal markers. Retinal whole mount preparations were made from albino rats in which three 0.1 µl injections of 2% FB were placed into the colliculus of one side and of 2% DY into the colliculus of the opposite side, on the day of birth. After survival times of 48 to 68 hrs a significant number of ganglion cells labeled from the ipsilateral colliculus was seen within all four retinal quadrants, with a markedly higher density in the lower temporal periphery. Only a very small proportion of the ipsilaterally projecting cells was doubly labeled.

A second series of animals injected in the same way was allowed to survive until postnatal day 12. In these cases the ganglion cells that were labeled from the ipsilateral colliculus were confined almost entirely to the lower temporal periphery as they were in the retinae of adult albino rats ipsilateral to a collicular injection made with either dye. In these cases very few doubly labeled cells were observed.

Our findings indicate that the ipsilateral retinocollicular projection is not formed by collaterals of axons that are directed to the contralateral colliculus. The postnatal restriction of the ipsilateral projection is due rather to the death of the ganglion cells that initially project to regions outside the rostromedial part of the ipsilateral colliculus, and not to collateral elimination.

Supported by NIH Grant EY-03653. J.W.F. is a Travelling Fellow of the U.K. M.R.C.

247.8 A ROLE FOR ELECTRICAL ACTIVITY IN THE RESTRICTION OF THE IPSILATERAL RETINOCOLLICULAR PROJECTION.
James W. Fawcett,* Dennis D.M. O'Leary, and W.M. Cowan, The Salk Institute, P.O. Box 85800, San Diego, CA 92138 (SPON: Francis Crick).

In adult rats the ipsilateral retinocollicular projection arises from a restricted population of ganglion cells in the lower temporal retina and is limited in its distribution to the rostromedial part of the superior colliculus. However, early in postnatal life it extends across the entire colliculus and arises from ganglion cells that are scattered across much of the retina. Removal of one eye shortly after birth prevents the restriction of the ipsilateral projection from the remaining eye (Land and Lund, Science, 205:698, 1979), which suggests that there is normally competition between the fibers from the two eyes.

We have confirmed (using the anterograde transport of WGA-HRP and TMB histochemistry) that early in postnatal life the ipsilateral retinocollicular projection in normal albino rats covers the entire colliculus, and that by postnatal day 12 (P-12) the projection is largely confined to its rostromedial part. In a second group of animals, enucleated on P-2, the remaining eye was injected with WGA-HRP; here labeled fibers were found to cover the entire colliculus on P-12.

In a third group of animals we injected 0.03 µl of 0.1% tetrodotoxin (TTX) into one or both eyes on every other day, from P-2 to P-10, in order to abolish optic fiber electrical activity. On P-11 WGA-HRP was injected into one eye, and the animals were killed on P-12. In animals with monocular TTX injections, in which the other eye was filled with WGA-HRP, the ipsilateral projection from this eye was found to persist over the entire colliculus, and was similar to that seen in neonatally enucleated animals. If the TTX injected eye was filled with WGA-HRP the ipsilateral projection was either completely absent or was restricted to the extreme rostromedial part of the colliculus. When both eyes were injected with TTX and either eye filled with WGA-HRP, the ipsilateral projection was distributed over the entire colliculus. In control animals in which the TTX treated eye was retrogradely labeled with WGA-HRP injected into the contralateral colliculus, we have been able to confirm that TTX treatment does not increase normal ganglion cell death; in these cases ganglion cell density was the same as in normal retinae.

Our findings indicate that the competition between optic fibers which normally leads to the preferential elimination of ganglion cells projecting to the ipsilateral colliculus is mediated by electrical activity in the competing fibers.

Supported by NIH Grant EY-03653. J.W.F. is a Travelling Fellow of the U.K. M.R.C.

247.9 SUPEROXIDE DISMUTASE-POLYETHYLENE GLYCOL (PEG-SOD) AS A FREE RADICAL SCAVENGER IN GERBIL CEREBRAL ISCHEMIA AND REFLOW.
†Joe S. Beckman* and §Charles J. Hannan, Jr.,(SPON: J.J. Bucccafusco), †Botany Dept., Duke University, Durham, NC 27706, and §Clinical Investigation Dept., DD Eisenhower Army Medical Center, Ft Gordon, GA 30905.

Preliminary findings, employing a 40 minute bilateral occlusion in gerbils, indicates a beneficial effect from a superoxide dismutase-polyethylene glycol preparation administered before occlusion. Superoxide dismutase (SOD) is a naturally occuring, ubiquitously distributed enzyme which plays a role in the protection of cells from oxygen toxicity. Free radicals of oxygen may be produced upon the reperfusion of ischemic tissue. SOD has been modified by bonding it to polyethylene glycol in order to increase its half life in plasma. The PEG-SOD prepared had an activity of about 2100 units/ml (assayed by inhibition of xanthine oxidase reduction of cytochrome c according to McCord and Fridovich, JBC 244: 6049, 1969). PEG-SOD (10 ul/gm or 20,000 units/Kg) was administered intraperitoneally to 20 animals and an equal volume of saline to a group of 13 control animals, 3 hours before occlusion. Mortality figures indicated a beneficial effect of PEG-SOD ($P = 0.0778$ by Chi Square). A clearance curve for PEG-SOD indicated plasma SOD activity to be considerably elevated at least 48 hours after injection.

These preliminary results are encouraging for a role of oxygen free radical damage during post ischemic reflow. Further studies to determine the exact time course for the protective effect as well as the role of vitamin E are now being conducted.

247.10 POSTNATAL DEVELOPMENT OF NEURONS IN THE OPTIC RADIATIONS OF THE CAT. D. M. Murakami*, G. J. Condo, and P. D. Wilson. (SPON: M. A. Baker) Dept. of Psychology, University of California, Riverside, CA 92521.

A population of neurons which may be transient during development was observed within the optic radiations of cats 1-6 weeks of age. These neurons could not be ascribed to the perigeniculate nucleus (PGN) or reticular nucleus of the thalamus (RNT). Corresponding regions in the adult cat did not reveal the same frequency and density of neurons as the younger kittens. In order to investigate the development and origin of these cells, intraocular injections of HRP were made in cats newborn to 52 weeks of age. Coronal sections through the dLGN were reacted with TMB and counterstained with thionin. No direct retinal projection to the PGN or RNT was observed at any developmental period. At birth through 4 weeks of age there is a high density of cells within the optic radiations between the PGN and RNT. The apparent number and density of these cells decreases after 4 weeks of age until 8 weeks when it becomes adultlike in density and frequency. Cells measured in the optic radiations reach a maximum diameter ($\bar{x}$=20.5μm) at 4-6 weeks of age and are circular in shape. By 8 weeks of age the cells are fusiform and smaller in diameter ($\bar{x}$=18.022μm). Morphological analyses of these cells in Golgi-Cox material indicates that they are clearly distinct from PGN neurons in that the optic radiation cells have a larger soma diameter, and 4-8 primary dendrites giving rise to a complex and relatively compact (200-249μm, $\bar{x}$=213μm) dendritic arbor, which is oriented perpendicular to the dLGN laminae.

Transsynaptic transport of WGA-HRP from intraocular injections were used to demonstrate projections of dLGN relay cells to the PGN and RNT. In adult cats HRP label was confined to regions near the somas and proximal dendrites of PGN cells, and was patchy to RNT neurons. No distinct label could be found in the region between the RNT and PGN. Cats 6 weeks of age and younger revealed broad and dense HRP label in the RNT. However, very little label could be found with the cells between the PGN and RNT typical of cats this young.

In conclusion, there is a distinct population of large round neurons between the PGN and RNT that do not receive visual input in young kittens. During the period of greatest maturation of the dLGN (4-8 weeks) a high proportion of these cells seem to disappear or migrate out of the region.

247.11 NEURAL TISSUE TRANSPLANTS RESCUE RUBROSPINAL NEURONS AFTER NEONATAL SPINAL CORD LESIONS. B.S. Bregman, Dept. Anatomy, Univ. Maryland, School of Medicine, Baltimore, MD 21201.

Spinal cord lesions sustained neonatally frequently result in massive retrograde loss of axotomized immature neurons. We have shown that implants of embryonic spinal cord survive, grow and differentiate when transplanted to the lesioned spinal cord in newborn animals (Bregman & Reier, SN Abstr, 1982). The current study was designed to determine whether red nucleus (RN) neurons which usually die after neonatal axotomy can survive in the presence of a graft of immature spinal cord. Spinal cord hemisections were made at T6 in 16 newborn rats, eight of which were recipients of fetal (E12-E14) spinal cord transplants. The other animals received no implant. At 30d to 1 year experimental animals and control (unoperated) littermates were sacrificed and the tissue blocks containing the RN were frozen and sectioned serially. A 1 in 5 series of 40um sections was collected in consecutive order and stained with cresyl violet. The volume of the RN and the number of neurons present were determined using a Zeiss MOP-30. In an additional series of animals, HRP-WGA was injected bilaterally into the lumbar spinal cord, caudal to the lesion or lesion and implant.

In implant recipients, labeled neurons were present in both RN, whereas, in hemisected animals without implants, only the RN ipsilateral to the lesion contained labeled neurons. The distribution of cells in the RN projecting to the intact spinal cord was identical with that in normal animals. The RN projecting to the side with implant contained labeled neurons in a more diffuse pattern, including some cells located in the dorsomedial portion of the nucleus. Cell counts indicate that neonatal spinal cord lesions result in the loss of approximately 45% of the neurons in the RN. ANOVA and Duncan's multiple range tests (α=0.05) indicate that there are significantly fewer neurons in the RN of animals with neonatal lesion alone ($\bar{x}$=2920.0) than in those with lesion and implant ($\bar{x}$=4790.6) or in control animals ($\bar{x}$=4444.3). Cell numbers in the control animals and those with implants were equivalent. The volume of the nucleus was decreased by 25% in animals with lesion alone but not in those with implants.

These results indicate that implants of fetal spinal cord can rescue immature axotomized RN neurons. At least some of these rescued neurons project caudal to the implant, as indicated by the retrograde HRP labeling. It is not clear whether these rescued neurons represent regenerating neurons, neurons sprouting rostral to the lesion, or later developing RST cells which are able to survive in the presence of the implant. The implant may provide a trophic influence necessary for cell survival that is absent after lesion alone.

Supported by: NIH Grants NS19259, NS13836 and Univ. Md. Bressler Research Fund.

247.12 AN EXAMINATION OF POSTNATAL NEURON DEATH IN THE LOCUS COERULEUS OF RATS. R. E. Ruth and S. K. Goldsmith. Inst. Study Dev. Disabil., Univ. Il. at Chicago, Chicago, IL 60608.

Naturally occurring neuronal death is one of the major steps in neurogenesis. In peripheral noradrenergic cells evidence indicates that cell death results from competition for a limited supply of nerve growth factor which, when retrogradely transported from the target to ganglion cells, promotes survival. The occurrence of cell death in central mammalian adrenergic neurons is less well documented. The rat locus coeruleus (lc) affords an opportunity to examine this phenomenon. The lc is relatively prominent at birth and all of its neurons are presumably norepinephrine-containing. Moreover these neurons cease proliferation and migration early in gestation. Therefore any decrease in cell number after birth would reflect the existence and magnitude of cell death.

Anesthetized rat pups were perfused with aldehydes on postnatal (P) days 1, 3, 6, 9, 12, 24, 40 or 120 days. Rate, volume and pressure of perfusate delivery varied as a predetermined function of age. Brains were embedded in paraffin, cut at 6um in sagittal or coronal planes and stained with thionin. Nucleolar counts were obtained from a 1-in-3 (P1,P3), 1-in-4 (P6—P12) or 1-in-5 (>P12) series through lc. Results presented here are derived from preliminary counts of P1 (n=5), P12 (n=3) and P40/P120 (n=2) brains. Each pup represented a separate litter; no correction factor was applied to the counts.

Light-microscopic features of the developing and adult lc were quite similar to previous descriptions (Sievers et al., 1981; Swanson, 1976). Particularly relevant for interpretting nucleolar counts, the size of lc neurons was smallest at P1 and largest at P12. By P40 neuronal size decreased slightly from the P12 maximum. Basophilia was also strongest at P12. Neuronal counts were actually lower and more variable at P1 (1724 195) than at P12 (1922 51) or P40/P120 (1840 28). Variations in neuronal counts across age were thus small and correlated with age-related variations in cell size, suggesting that thinner sections at P1 and P40 would yield higher estimates of cell number. In any event there is little evidence thus far to suggest that natural cell death occurs to any great extent in the postnatal rat lc. More firm conclusions await examination of the remaining tissue.

247.13 EFFECTS OF UNDERNUTRITION ON THE NUCLEUS RAPHE DORSALIS AND NUCLEUS LOCUS COERULEUS: A GOLGI MORPHOMETRIC STUDY IN RATS OF THREE AGE GROUPS. S. Diaz-Cintra, L. Cintra, T. Kemper* and P.J. Morgane. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545 and Instituto de Investigaciones Biomedicas. UNAM, Dpto. de Fisiology, C.P. 04510 Mexico, D.F.

Using morphometric analysis on cells of the nucleus raphe dorsalis and nucleus locus coeruleus in control and undernourished animals at 30, 90 and 220 days, we found in both nuclei similar types of cells: fusiform, multipolar and ovoid (Brain Res. 207:1-16, 1981; Brain Res. 247:17-28, 1982). In control animals all three cell types showed synchronous age-related changes in spine densities that in the two nuclei were out-of-phase with each other (Develop. Brain Res. 4: 487-490, 1982). In the nucleus raphe dorsalis protein deprived rats failed to show a period of proliferation and elimination of dendritic spines seen in controls resulting in a spine deficit at 90 days of age (Brain Res. 221: 243-255, 1981). In contrast, in the 8% casein diet rats the three cell types in the nucleus locus coeruleus more closely followed age-related decreases and increases in spine density seen in the controls. When the age-related changes in the nucleus locus coeruleus and nucleus raphe dorsalis in the protein deprived rats are plotted together it can be seen that the direction of the age-related changes are similar in the two nuclei. Thus, the out-of-phase relationship seen in the controls in these two nuclei fails to occur in 8% casein diet rats. Whether this is due to a failure of the reciprocal inhibition between these two nuclei or from some other mechanism cannot be answered at this time. It does suggest, however, a closer synchrony of the synaptic relationships between these two nuclei in the protein undernourished rat. Whatever the mechanism, it is clear that the relationship between these two nuclei appears to be fundamentally different in the 8% casein diet rats as compared to controls. Studies are in progress to determine how the altered interrelationships between these two biogenic amine nuclei may affect function in forebrain areas to which these nuclei project. (Supported by NIH Grant HD-06364).

247.14 CELL COUNTS IN THE INFERIOR OLIVARY NUCLEUS AND SYNAPSE ELIMINATION IN THE DEVELOPING OLIVO-CEREBELLAR SYSTEM OF RODENTS.
J. Mariani, N. Delhaye-Bouchaud, B. Geoffroy and H. Shojaeian, Dept of Molecular Biology, Pasteur Institute and Lab. Neurophysiologie Ontogénétique, Univ. P. et M. Curie, Paris, France.

In the developing rodent cerebellum, the multiple innervation of Purkinje cells (PCs) by climbing fibers (CFs) is maximal on postnatal (p.n.) day 5 and decreases thereafter until the adult mono-innervation is attained around p.n. day 15. Is this regression related to the withdrawal of redundant CF collaterals or to neuronal death in the inferior olivary nucleus (I.O.N.), which is the unique source of CFs in rodents ?

To answer this question, cells in the different I.O.N. subnuclei were counted in serial frontal sections of the medulla oblongata in developing rats from birth to adulthood. Appropriate corrections for double counting were applied. The total number of cells per-olive decreased from about 27,000 on day zero to about 21,000 on p.n. day 5. An apparent increase in the cell number occured subsequently and values in the adult were grossly similar to those found on day zero. The fraction of total cells represented by each different subnucleus remained approximately constant throughout this evolution. If no correction was applied the same biphasic evolution was observed. A similar postnatal increase in cell counts was observed in mice for the I.O.N. (Caddy and Biscoe Phil. Roy. Soc. Lond. 1979, 287, 167-201) and the PCs (Diglio and Herrup, Neurosc. Abs. 636, 1982). No conclusive explanation is yet available for this apparent increase since it is generally admitted that the last division of precursors of IO neurons occurs prenatally (Altman and Bayer, J. Comp. Neur. 179, 49-76, 1978).

Additional data were obtained by counting the IO population in adult mutant mice (weaver and staggerer) in which the multiple innervation of PCs is maintained. No significant difference was noted compared to control littermates of the same age.

In conclusion, this study reveals that cell loss occurs in the I.O.N. of the rat after birth. Nevertheless, it takes place before the peak of multiple innervation is attained and affects only 20% of total cells. Taken to-gether with the data obtained in adult mutant mice this suggests that neuronal cell death does not play a major role if any during elimination of redundant synapses between climbing fibers and Purkinje cells.

Supported by C.N.R.S., grant n° 955139

247.15 GOLDFISH STROBOSCOPIC ILLUMINATION: REGENERATION IN A FLASH FAILS TO SHARPEN THE RETINOTECTAL MAP.
J.T. Schmidt and L.E. Eisele*, Dept. Biol. Sci., SUNY Albany, Albany, NY 12222.

Using intraocular injections of tetrodotoxin (TTX) to block spike activity, we previously showed that the regenerating retinotectal map goes through an activity-dependent sharpening process during the time of initial synaptogenesis (20-34 days postcrush). Without activity the map was normally organized but the multiunit receptive fields at each point were grossly enlarged (25 to 40 degrees versus 11 normally). Single unit receptive field sizes were not enlarged. By 35 days the maps formed were relatively stable; that is, normal maps stay normal in spite of blockade of activity, and abnormal maps stay abnormal in spite of release from blockade for months. Therefore we have termed the 20-35 day period a sensitive period. Our working hypothesis is that neighboring ganglion cells, which are known to fire in a statistically correlated fashion, might use this relative synchrony of activity to eliminate inappropriate branches of an initially diffuse arbor and concentrate in one area. Within this area, their EPSP's would summate, perhaps leading to a strengthening of those synapses. If such a model is correct, then sharpening could also be blocked 1) if activity were allowed but all ganglion cells were made to fire synchronously (strobe light) or 2) if activity were allowed presynaptically but postsynaptic transmission were blocked by alpha-Bungarotoxin, which binds to nicotinic cholinergic receptors on tectal cells.

In the strobe rearing experiment, fish were placed in a white featureless environment inside a light tight box, strobed at 1/sec from day 20 onwards, and recorded at 35 to 90 days. All maps showed the same gross enlargement of the multiunit receptive fields as seen in the TTX fish, averaging 36 degrees vs 28 for TTX. Thus errors in targeting of the regenerating arbors were not eliminated when all ganglion cells fired in synchrony. This implies that the TTX results were not due to deleterious effects on ganglion cells, but to the elimination of cues in the spatiotemporal pattern of their activity.

In the alpha-Bungarotoxin experiments, an osmotic minipump was attached to the fish's head and a canula delivered 2-3 microliters of micromolar toxin per day from day 20 to 39 after nerve crush. Again the multiunit receptive fields were greatly enlarged, averaging 30 degrees vs 28 for TTX. Controls infused with Ringers were normal. We conclude that the elimination of misdirected branches of the initially diffuse arbors procedes via a mechanism whereby neighboring ganglion cells firing in relative synchrony stabilize each other's synaptic connections through summation of their postsynaptic potentials. (Supported by NIH grant EY 03736 and a Sloan Foundation Fellowship to J.T.S.).

247.16 SYNAPTIC PROPERTIES OF MOTOR UNITS IN THE FROG CUTANEOUS PECTORIS MUSCLE.
L.O. Trussell* (SPON: A.D. Grinnell). Dept. of Biology, UCLA, Los Angeles, CA 90024.

The size, distribution, and synaptic physiology of motor units have been examined in frog cutaneous pectoris (c.p.) muscles. Unlike the frog sartorius (Grinnell & Trussell, 1983, J. Physiol., in press), very small motor units with high tetanus-to-twitch tension ratios were rare, consistent with the c.p. synapses' generally higher quantal content and suprathreshold operation (Grinnell & Herrera, 1980, J. Physiol., 307: 301-317). Nevertheless, motor unit tetanic tensions varied over a 30-fold range. Mapping of the distribution of synapses within motor units electrophysiologically and by HRP uptake revealed that single axons could innervate fibers throughout the entire muscle, showing little tendency towards a geographic localization of their innervation field.

EPPs from identified motor units in curarized muscles were examined to see if motor unit size and synaptic strength are directly related, as they are in the sartorius. Mean EPP size within motor units decreased with motor unit size, showing a 6-fold change over the range of units examined. Examination of the range of muscle fiber input resistances between units showed that small units were associated, in general, with smaller fibers. Thus, the differences observed in mean EPP amplitude with motor unit size must reflect even larger differences in quantal release.

While the mean EPP and fiber sizes of motor units varied with motor unit size, in many cases a marked variability was observed in the EPP, input resistance, and diameter of singly innervated fibers within the same unit. This variability in EPP size did not parallel fiber size differences. Thus, both transmitter release and the extent of growth of fibers may be regulated by more than just the identity and behavior of the innervating axon alone. Among the synapses formed by a given motor axon, the weakest inputs were generally members of a polyneuronally innervated junction. For smaller units, such complex junctions often appeared on the largest fibers in the motor unit, suggesting the overlap of small and large units, and the larger units' dominance in determining fiber properties.

These data suggest that, in the adult frog, motor neurons appear to differ in the mean level of transmitter release of their synapses, perhaps reflecting intrinsic differences in their ability to support synapses. Variation within a motor unit may be the result of competitive influences during and following the period of synapse elimination.

(Supported by USPHS research grant NS 06232 and a grant from the Muscular Dystrophy Association.)

248.1 ULTRASTRUCTURAL LOCALIZATION OF SEROTONIN IN THE HIPPOCAMPAL FORMATION FOLLOWING RAPHE IMPLANTS IN NEONATAL RATS. V.R. Holets and C.W. Cotman. Department of Psychobiology, University of California, Irvine, CA 92717.

Fetal raphe (serotoninergic) neurons implanted into neonatal rats will reinnervate the developing hippocampus and dentate gyrus. However, the serotonin (5-HT) fibers do not form a laminated pattern as in normal animals, but hyperinnervate the entire hippocampal formation. The 5-HT immunoreactivity in the hippocampal formation appears as long, varicose fibers with enlargements that can be seen at the light microscopic level. Using the peroxidase, antiperoxidase (PAP) method we have identified 5-HT immunoreactivity at the ultrastructural level in the hippocampus and dentate gyrus in normal rats and raphe implant rats.

The entorhinal cortex was ablated and the fimbria cut in 3 day old neonate rats. The raphe nuclear area from rats of embryonic age 16-19 days was implanted into the entorhinal cortex site. Normal adult rats (60 days old) and rats which received lesions and implants (6 days post-implant) were perfused intravascularly with buffered 4% paraformaldehyde-0.2% glutaraldehyde. Vibratome sections (100 μm) were cut and processed for immunohistochemistry using the PAP procedure by Sternberger. Rabbit, anti-5-HT antiserum (supplied by Dr. Robert Elde, Univ. of Minnesota) was used at a dilution of 1/1000. The sections were then osmicated, dehydrated and embedded in Spurr's resin. Specificity of staining was determined using antiserum pretreated with 5-HT. The absorption controls indicated that the immunoreactivity observed was specific for 5-HT.

At the electron microscopic level, the morphology of the 5-HT varicosities was similar throughout the hippocampal formation in normal rats (ranging from 0.5-3 μm). In rats with raphe implants, the 5-HT immunoreactive varicosities were more frequent and large (0.6-5 μm) varicosities were observed. The 5-HT immunoreactive varicosities in the hilus and infragranular zone of the dentate gyrus of both normal and raphe implant animals measured 0.6-2.0 μm, and contained round clear vesicles (40-50 nm) and dense core vesicles. The 5-HT immunoreactivity appeared to be mainly associated with vesicles in the varicosities. Although the 5-HT immunoreactive varicosities were found to be distributed throughout the neuropil of the hippocampal formation in raphe implant rats and throughout certain areas of the normal rat (ie, hilus and molecular layer), they were infrequently observed to form synatic contacts with neuronal structures.

Supported by NIH Fellowship NS07007 (VRH) and NIMH Grant MH19691 (CWC).

248.2 PLASTICITY OF NORADRENERGIC INNERVATION OF THE SUPRAOPTIC NUCLEUS FOLLOWING LESIONING. B.J. Davis, C.D. Sladek and J.R. Sladek, Jr. Department of Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, N.Y. 14642.

Vasopressin (VP)- and oxytocin (OX)-containing magnocellular neurons of the supraoptic nucleus (SON) are densely innervated by noradrenergic (NE) fibers that originate primarily in the A1 catecholamine cell group. NE input reaches the SON via the medial forebrain bundle (MFB) and the supraoptic decussations (SOD).

During the course of an ongoing investigation of plasticity of functional interactions of NE fibers and their VP target neurons, we found that bilateral lesions of the MFB and SOD, resulting in complete denervation of NE input to the SON, led to adipsia that was followed by death within 7-10 days. In contrast, partial denervation of NE input to the SON led to slight initial deficits of water intake that eventually recovered to normal levels. The purpose of the present investigation was to provide a morphological correlate of this apparent functional plasticity by evaluating regenerative responses of NE fibers in the SON following partial denervation.

Adult male rats were given unilateral intracerebral injections of 6-hydroxydopamine (6 ug in 1.5 ul ascorbic acid vehicle) into the MFB immediately caudal to the SON. Animals were killed after 4, 7, 10, 14, 21 or 35 days and brains were processed for formaldehyde-induced fluorescence. The pattern of NE varicosities in the SON on the lesioned side was compared with that of the intact side and with the SON of unoperated controls.

Four days following lesioning, fibers of the MFB appeared swollen, tortuous and brightly fluorescent, thus exhibiting morphology which is typical of damaged catecholamine axons. There was a marked reduction of NE varicosities in the SON ipsilateral to the lesion. Denervation of magnocellular perikarya was most pronounced in the lateral portion of the SON. The NE innervation pattern in the SON contralateral to the lesion appeared qualitatively similar to that of unoperated controls. At 7 and 10 days fine, delicate processes appeared to emanate from swollen profiles in the MFB. Varicose fibers also were seen crossing the lesion site, but they could not be traced into the SON. Swollen, brightly fluorescent varicosities were observed adjacent to magnocellular neurons within the SON. These reactive fibers, which appeared to be confined to the lateral portion of the SON, were increased in number at 14 and 21 days. By 35 days post-lesion the ipsilateral SON exhibited a striking increase in the number of varicosities with respect to the intact SON. These results suggest that partial denervation of NE afferent fibers to the SON led to an apparent hyperinnervation of their magnocellular target neurons. Moreover, they provide a morphologic basis for recovery from functional deficits following partial denervation of NE input to the SON.

Supported by R01 NS 15816 and F32 MH 08811.

248.3 **EFFECTS OF BLOCKING GABA ON HIPPOCAMPAL CA1 INHIBITION & PLASTICITY.** **C.Hendricks & T.J.Teyler. Neurobiology Dept., NE Ohio Coll Med, Rootstown, OH, 44272.**

GABA applied to CA1 cell soma appears related to basket cell recurrent inhibition, while dendritic GABA responses are quite different and may result from a feed-forward inhibitory mechanism (Alger & Nicoll, 1982; Andersen, etal, 1980). The present group of experiments: (1) characterized the effects of blocking GABA on different CA1 inhibitory responses and (2) determined what effects this disinhibition has on hippocampal plasticity.

The GABA blocking agent bicuculline (BIC) was applied to pyramidal cell soma or the mid-radiatum region of hippocampal slices via iontophoresis or pressure ejection. Using the paired-pulse method, inhibitory responses of the second pulse were titrated away with BIC. After titration was complete, tetanic stimulation (100Hz, 1sec) was given to the radiatum.

Inhibition was characterized by pairing stimuli from several different inputs (SC=Schaffer collaterals, OR=oriens, ANTI=alveus-antidromic). BIC ejected at pyramidal cell soma caused a strong disinhibition of what appeared to be recurrent inhibitory fibers. The second pulse of all pairings tested (SC-SC, OR-SC, OR-OR & ANTI-SC) lost inhibitory responses and single population spikes became multiple spikes, although they did not become larger in amplitude. Tetanus after this treatment sometimes resulted in potentiation of the stimulated fibers, but many times resulted in seizure activity with resulting postictal depression.

Application of BIC to the SC synaptic region caused strikingly different results. Inhibition of the SC-SC could be titrated out, while OR-SC and ANTI-SC inhibition was left intact. This indicated that BIC was localized to SC synapses, since the above cell soma response was not observed. Tetanic stimulation given to the SC in this condition not only potentiated the SC responses but also potentiated OR responses. LTP was pronounced and lasted over 1 hour in most cases observed, EPSP thresholds were unchanged. The results indicated local circuit interactions are more complex than present models of hippocampal function. These results also suggest that local inhibitory neurons probably play some role in modulating hippocampal plasticity, although they may not be responsible for LTP. (Supported by NSF)

248.4 CROSS-SPECIES SEPTAL TRANSPLANTS: CHOLINERGIC ENHANCEMENT OF T-MAZE PERFORMANCE. R.P. Bodony*, J.K. Daniloff, W.C. Low, and J. Wells (SPON: J. Held), Departments of Anatomy and Neurobiology, and Physiology and Biophysics, University of Vermont, Burlington, VT 05405

Both cholinergic graft and cell suspension transplants from small rat embryos restored T-maze behavior in rats subjected to bilateral fornix transection. Behavioral recovery correlated significantly with the extent of the transplant growth into the deafferented hippocampus (Dunnett, et al., Brain Res., 251: 335, 1982). Dopaminergic cross-species grafts induced a compensation to a turning response generated by a nigrostriatal lesion (Björklund, et al., Nature, 298: 652, 1982). Previously, we characterized the growth of fibers from cholinergic cell suspensions transplanted across species (Daniloff, et al., Anat. Rec., 205: 43A, 1983). The present experiment explores the ability of of xenogenic cell suspension transplants from the embryonic septum to restore T-maze behavior.

Three groups of Sprague-Dawley rats received bilateral operations: a sham group, a group with a fornix-fimbria lesion, and a lesioned group which received bilateral cell suspension transplants from C-57 Bl/6J mouse embryos. Beginning one month after surgery, animals were conditioned daily for a food pellet reward on an elevated T-maze for 3 weeks. After the conditioning period, performance testing was conducted daily for 9 weeks. For each trial, the animal was forced to choose one arm of the T-maze for its reward on the first run; immediately it was allowed a free choice on the second run. The correct response on the second run was to select the alternate arm for its reward. The number of times, in six daily trials, the animal alternated correctly on the second run was recorded. The animals were sacrificed and brain tissue was stained for acetylcholinesterase (AChE).

Sham animals stabilized at nearly 100% correct responses after 3 weeks. In contrast, animals with complete lesions showed little improvement above the initial 50% correct responses even after 9 weeks of testing. The transplant group steadily increased their performance after 3 weeks and performed at near normal levels (90% correct) after 9 weeks. These data are very similar to those reported for homogenic transplants (Dunnett, et al., 1982).

The relationship between AChE fibrous ingrowth and behavior will be discussed. The present results indicated that xenogenic septal cell suspension transplants can (1) restore part of the complex behaviors altered through hippocampal denervation and (2) in a manner similar to homogenic transplants.

Research supported by PHS #5429-17-3

248.5 ULTRASTRUCTURAL X-RAY MICROANALYSIS OF HIPPOCAMPAL ACETYLCHOLINESTERASE ACTIVITY DERIVED FROM SEPTAL TRANSPLANTS. W.C. Low[*1], P.R. Lewis[*1], S.T. Bunch[*2], A. Björklund[*4], U. Stenevi[*4], S.B. Dunnett[*3], and S.D. Iversen[*3]. [1]Dept. of Physiology and Biophysics, Univ. of Vermont, Burlington, VT, [2]Physiological Laboratory and [3]Experimental Psychology Laboratory, Univ. of Cambridge, U.K., and [4]Dept. of Histology, Univ. of Lund, Sweden.

Recent experiments have demonstrated that transplanted embryonic septal neurons are capable of reinnervating the hippocampal formation of adult rats. Transplant-derived acetylcholinesterase (AChE) activity was examined within ultrastructural compartments of the hippocampal formation using methods of electronmicroscopy in conjunction with electron probe X-ray microanalysis. Tissue taken from the septal-diagonal band region of fetal rat pups 15-17 days of gestation were graffed into a cavity overlying the anterior thalamus and rostral to the hippocampal formation. The cavity for implantation was created by unilateral aspiration of the fornix-fimbria which also eliminated the intrinsic cholinergic septal projection to the hippocampus. The contralateral septal projection to the hippocampal formation served as the control. Reinnervation of the hippocampal formation by transplant-dervied fibers was examined with AChE histochemistry. The pattern of staining in tissue reinnervated by the transplant was similar to the contralateral control tissue as seen at the level of the light microscope. The intensity of staining, however, was markedly reduced. At the ultrastructural level clusters of AChE reaction products (copper and sulfur) were observed in regions of the hippocampal formation which correlated with the staining seen at the light microscope level. There appeared to be fewer clusters in these regions in transplant-derived preparations, however, in comparison to contralateral controls. Electron probe X-ray microanalysis of these compartments revealed that the number of copper and sulfur X-rays emitted were similar for transplant-derived and control tissue. These results suggest that the enzymatic activity of AChE derived from septal transplants is the same as that in the contralateral control tissue, and that the reduction in transplant-derived AChE staining as seen at the level of the light microscope is most likely due to a decrease in the number of AChE containing fibers and/or a decrease in the compartments of AChE contained within each fiber.

248.6 THE ABILITY OF DAY 18 FETAL STRIATAL IMPLANTS TO REVERSE THE LONG TERM LOCOMOTOR ABNORMALITIES IN THE KAINIC ACID RAT MODEL OF HUNTINGTON'S DISEASE.
A.W. Deckel*, R.G. Robinson and P.R. Sanberg (SPON: D. Newman).

Kainic acid (KA) lesions of the striatum have been shown to produce biochemical, neuropathological, and behavioral deficits in rats similar to those seen in humans with Huntington's Disease. Recently, transplantation of fetal striatal tissue has been shown to survive in KA lesion rat striatal tissue. This experiment assessed the ability of these transplants to mediate behavior within the host brain of rats with previous KA lesions and reverse the long term locomotor deficits.

Twenty-one adult female Sprague-Dawley rats were assigned to one of three groups (7 per group), including lesion only (LO), lesion and transplant (LT), and control (CO) groups. Rats in the LO and LT groups received bilateral KA injections (0.8ug/0.4ul per side) in the anterior medial striatum. Seven days later, the LT rats received bilateral implants of day 18 fetal rat striatum directly into the KA lesion host striatum. The LO rats underwent the same surgical procedure as transplanted animals, but did not receive fetal striatal implants. The CO group received sham lesions and sham transplant surgical procedures. Twelve weeks later all animals were placed individually in computerized Animal Activity Monitors (Omnitech Electronics) and tested over 12 ten minute periods. These monitors provided detailed infomation of the animal's pattern of locomotion using 21 different variables measuring activity in the horizontal and vertical planes. A repeated measures ANOVA was used to analyze the statistical significance of the data.

The results demonstrated that although there were no differences between groups during the first period, the LO rats were significantly more active over the remaining eleven test periods than controls on 9 measures of activity (eg. total distance, movement time, etc.). This pattern of activity suggested that there was a slower rate of habituation by the LO group compared to CO. The LT group showed rates of habituation similar to the CO group, and appeared recovered from the habituation deficit shown by the LO group.

These findings suggest that the fetal implants will mediate behavior within the host brain, and reverse the locomotor deficits exhibited by the LO group. This procedure may provide a treatment for the long term behavioral disorders found in this animal model of Huntington's Disease. Supported by the Research Scientist Development Award (RGR) MH00163, NS15178, NS18622, by the Pratt Family and Friends Huntington's Disease grant, and Tourette Syndrome Association.

248.7 INTRAHIPPOCAMPAL INJECTIONS OF CYCLOHEXAMIDE REVERSIBLY BLOCK LONG TERM POTENTIATION. O. Steward and S. Brassel. Depts. of Neurosurgery and Physiology, Univ. of Va. Sch. of Med. Charlottesville,VA 22908.

Recent studies have suggested a relationship between long-term potentiation (LTP) in the dentate gyrus and protein synthesis. Duffy et al. (Sci. 212, 1148-1151, 1981) report an increased release of newly synthesized protein by hippocampal slices after the induction of LTP. Fifkova et al. (J. Neurocytol. 11, 183-210) report that peripheral administration of anisomycin (a protein sythesis inhibitor) suppressed the changes in spine size which accompany LTP. To date, however, there have been no studies of whether protein synthesis inhibitors affect the increases in synaptic potency which represent LTP.

Animals were prepared for acute neurophysiological recording with stimulating electrodes positioned bilaterally in the entorhinal cortex, and recording electrodes positioned in the dentate gyrus. A 1µl Hamilton microsyringe filled with 10µg of cycloheximide in 1µl of saline was positioned within 1mm of one of the recording electrodes. The stimulus intensities were adjusted so as to obtain approximately equivalent responses on each side; potentiating stimulation which results in similar response amplitudes usually leads to similar levels of LTP. Baseline responses were recorded for 30 minutes; the cyclohexamide was injected over a 5 minute interval; and a 30 minute post-injection baseline was obtained. Potentiating stimulation was delivered to each pathway (ipsilateral and contralateral to the cyclohexamide treatment) 30 and 90 minutes post-injection.

LTP was consistently observed on the control side, but was reduced or eliminated in the cyclohexamide-treated dentate gyrus when the potentiating stimulation was delivered 30 minutes post-injection. In some animals with complete suppression of LTP, there was no change in the baseline responses prior to potentiation. In other animals, where the recording electrode and the syringe were quite close, the pre-potentiation baseline responses were altered, in that the amplitude of the population spike increased. By 90 minutes post-injection, LTP could be induced in the cyclohexamide treated dentate gyrus to a level comparable to the control side.

LTP seems to require ongoing protein synthesis in the dentate gyrus. We propose that the process requires proteins which are produced in the postsynaptic cell, perhaps protein produced by the polyribosomes which are selectively localized under spine bases (see Steward & Levy, J. Neurosci. 2, 284-291, 1982). It is possible that LTP induces the synthesis of some protein, or that protein which is crucial for LTP turns over rapidly, and must be continually replaced by protein synthesis.
Supported by NSF Grant #BNS80-21865 & RCDA #NS00325 to O.S.

248.8 THE PLASTICITY OF SPECIALIZED SYNAPSES AND DIRECT CELL-CELL APPOSITION IN RAT SUPRAOPTIC NUCLEUS. Charles D. Tweedle and Glenn I. Hatton, Dept. of Anatomy and Neuroscience Program, Michigan State University, East Lansing, MI 48824.

Water deprivation for short periods of time (4-24 h) and parturition have been shown to dramatically increase the amount of direct soma-somatic contact between magnocellular neurosecretory cells (MNC's) of the adult rat supraoptic nucleus (SON). This increased contact is apparently by means of the withdrawal of thin astrocytic glial processes from between the MNC somata. It has been shown that more chronic stimulation of the SON induced by suckling or drinking 2% saline for 10 days leads not only to an increase in soma-somatic apposition, but also to the appearance of specialized "double" synapses (with one pre-synaptic terminal ending on 2 adjacent MNC somata). Such double synapses are extremely rare in the SON of control animals (Neurosci. 1981, 6, 919-930; Brain Res. Bull. 1982, 8, 197-204; Neurosci. Abstr. 1982, 8, 745). An experiment was designed to assess whether these specialized synapses, once formed, would disappear when the SON neurons were no longer chronically activated.

Young adult (100 day old) male and female rats were maintained 10 days on 2% NaCl instead of drinking water. Other rats were likewise maintained on saline for 10 days and then allowed access to drinking water again for 5 or 14 days. Thin sections from the saline-treated and control rats were quantitatively examined at the ultrastructural level. 1.9% of SON cells were in direct contact in control animals and 28.9% in the 10 day saline-treated group. 1.2% of MNC's showed double synapses in the SON of the control animals compared to 20.4% in the 10 day saline-treated group. In the animals rehydrated for 5 days the amount of SON cell apposition was back to control levels, but the number of double synapses was still elevated. In the animals rehydrated for 14 days only 2.4% of the MNC cells were contacted by double synapses. There were no sex differences seen in the results from these experiments.

It thus appears that not only can chronic activation of MNC's induce the appearance of new synapses in the SON, but that, with time, these synapses can also be eliminated when the stimulation ceases. Our working hypothesis is that the changes in cell-cell apposition by glial movement may be integral to the changes in synapse number and that both the changes in cell apposition and synapses are physiologically meaningful adaptations of the SON. (Supported by NIH Grant NS 09140).

248.9 PLASTICITY IN SUPRAOPTIC NEURONS: DENDRITIC BUNDLES AND DOUBLE SYNAPSES VARY WITH HORMONE DEMAND. L. S. Perlmutter, C. D. Tweedle and G. I. Hatton. Dept of Psychology, Anatomy and Neuroscience Program, Michigan State University, E. Lansing, MI 48824.

Magnocellular neurosecretory cells (MNCs) of the supraoptic nucleus (SON), which produce and release oxytocin, display a burst of activity that is proportional to the amount of oxytocin released in lactating rats (J. Physiol., 1975, 250, 443-461). Increases in the amount of soma-somatic apposition in post-partum and lactating rats and the percentage of MNC somata in lactating rats with double synapses (i.e., one presynaptic terminal making contact with two postsynaptic neurons) have also been found (Brain Res. Bull., 1982, 8, 197-204). These morphological changes may be involved in synchronizing the burst activity of the MNCs. Dendritic bundles (i.e., several dendrites in direct apposition) have been reported in several locations within the CNS, and it has been postulated that these bundles may serve to influence rhythmic activity (Golgi Centennial Symposium, Raven Press, 1975, 347-354). We now report that the presence of dendritic bundles in the SON, as well as the number of double synapses onto these dendrites, vary with hormone demand.

Four young adult rats from each of the following experimental conditions were used: Virgin females, pre-partum (day 21 of gestation), post-partum (2-24 hrs after delivery; litter size 4-13 pups), and lactating (for 14 days with a minimum of 6 pups). Rats were perfused transcardially, the SONs removed and prepared for electron microscopy morphometric analysis.

There was a significant difference in the average number of dendrites per bundle across the four groups ($H = 10.47$, $p < .02$). Planned comparisons using the Mann-Whitney U test revealed that virgins had a significantly smaller average number of dendrites per bundle than the three other groups, as did pre-partum compared with post-partum ($p < .02$ for each comparison). Further, the ratio of double synapses onto these dendrites to the total number of dendrites varied with treatment group ($H = 9.15$, $p < .05$). Comparisons revealed a significant increase in double synapses in post-partum compared with virgins ($p < .02$) and with pre-partum rats ($p < .02$). The total number of synapses per dendritic profile did not vary across conditions.

Formation of dendritic bundles and the increased occurrence of double synapses may be additional mechanisms to synchronize neuronal activity. The greatest change was found in the post-partum rats, suggesting a role in the rapid rise of oxytocin release immediately preceding parturition. The less extreme changes observed in the lactating rats indicate that these adaptations are reversible. Hence, rapidly occurring plasticity has been confirmed at the dendrite as well as at the soma of supraoptic MNCs.

Supported by NIH Grant NS09140.

248.10 TIME COURSE OF SYNAPSE REPLACEMENT IN THE MOUSE HIPPOCAMPAL DENTATE GYRUS. K.J. Anderson,* S.W. Scheff and S.T. DeKosky. Depts. of Anatomy and Neurology, Univ. of Kentucky and V.A. Medical Center, Lexington, KY 40536.

Our laboratory has focused on fundamental morphological changes in the central nervous system following damage. Specifically we have monitored the loss and reacquisition of synapses in the rat hippocampal formation. A unilateral ablation of the entorhinal cortex results in a major deafferentation of the granule cell dendritic field in the hippocampal dentate gyrus. Residual afferents to the dentate molecular layer are responsible for the reinnervation of this area, and play a role in the return of synaptic density to pre-operative levels. In the adult rat, return of synaptic contacts and removal of degenerative debris follow a strict time course. In other species it is not clear whether replacement of lost synaptic contacts is restricted to similar temporal parameters and involves the same reactive afferents. We have chosen to examine the process of axon sprouting and reactive synaptic replacement in a commonly used strain of mouse, the C57 black.

Thirty-five adult mice were subjected to a unilateral removal of the entorhinal cortex and allowed to survive for 2, 4, 6, 10, 15, 30, or 60 days following surgery. The hippocampi from these mice were fixed and processed for either light or electron microscopy. Sections for light microscopic study were stained either with the Holmes fiber method to assess changes in the commissural-associational fiber plexus or for AChE histochemistry to examine alterations in the septohippocampal afferents. Tissue for electron microscopy was processed by standard techniques and ultrathin sections encompassing the dentate gyrus molecular layer were cut. Photomontages were constructed from these sections that extended from the dorsal layer of the granule cells to the hippocampal fissure. All recognizable synapses were classified as either intact or degenerating and expressed as the number of synapses per 100 um^2. At this time, our preliminary results show that an entorhinal lesion initially reduces the synaptic density of the outer molecular layer to 7% of control. By 15 days post-lesion, synaptic density reaches 30% of control while at 30 days post-lesion, the synaptic density is restored to 50% of control. The course of the sprouting response in mice will be related to changes known to occur in the adult rat.

(Supported by NIH grants NS16981, NS00444, and the V.A. Medical Research Service.)

248.11 SURVIVAL OF ADULT MOUSE RETINAL GANGLION CELLS FOLLOWING INTRACRANIAL OPTIC NERVE TRANSECTION. M.R. Moore[1], R. Madison[2], and R. L. Sidman[2]. Yale Med. Sch.[1], Departments of Neuroscience and Neuropathology, Childrens Hosp. and Harvard Medical School[2], Boston, MA. 02115

Several laboratories have recently demonstrated a population of optic nerve axons and cells in the retinal ganglion cell layer which survive intracranial transection in the rat or mouse (see also Madison et.al., this volume). The following study was undertaken to determine if specific manipulations to the transected adult mouse optic nerve would affect the number or size class of cells surviving in the retinal ganglion cell layer. We tested the effects of: 1) intraorbital or 2) intracrainial optic nerve transection, or 3) intracranial transection with grafting of a short segment of either autologous sciatic nerve, or 4) intracrainial transection and grafting of a heterologous segment of neonatal optic nerve.

At the end of a 7-9 month postoperative survival time, animals were anesthetized and perfused with 20 ml 0.9% saline followed by 100 ml 20% formalin/saline. Retinal wholemounts were prepared from both transected and non-transected sides. Data were collected from retinal wholemounts with a computer-controlled light microscope. Cell size and location were entered on-line from a digitizing tablet to a display terminal and then to a VAX-11/780 computer for further analysis. The wholemount was divided into inner, middle and outer zones. Zone boundaries were established as 1/3 and 2/3 the radial distance from the optic nerve head to the wholemount outer border. Each zone was then divided into equal grid squares that were tabulated randomly at a final magnification of 1600 X. Approximately 1-2% of the total area of each retina was quantified.

Contralateral control retinal cell densities were 10.98 ± 1.65 (inner), 10.16 ± 1.3 (middle), and 8.06 ± 0.20 (outer); expressed as $10^3/mm^2$ of retinal area, mean ± SEM. Intraorbital transection resulted in complete necrosis of the retina within 10 days. Nine months after intracranial transection alone there are; 4.23 ± 0.34 (inner), 4.48 ± 0.40 (middle), and 4.25 ± 0.54 (outer). Both autologous sciatic nerve (N=2) or heterologous neonatal optic nerve (N=2) grafts are equally successful in allowing more cells to survive at 7 months after surgery; 7.98 ± 0.40 (inner), 6.70 ± 0.27 (middle), and 5.11 ± 0.09 (outer); $p<.01$ compared to intracranial transection alone, each and every zone. Under these processing conditions, many of the cells that are >40 μs in the mouse retina are believed to be ganglion cells. With intracranial transection alone approximately 10% or 0.43 ± 0.08 of cells >40 μs survive, compared to 17% or 0.69 ± 0.02 with the grafts; $p<.01$. Supported by NIH grants NS14768, N007017, and HD06276.

248.12 B-ADRENERGIC RECEPTOR REGULATION OF LONG-TERM POTENTIATION IN THE HIPPOCAMPUS. William F. Hopkins* and Daniel Johnston. Neuroscience Program, Baylor College of Medicine, Houston, TX 77030.

We have previously demonstrated that bath-applied norepinephrine (NE,1-10 uM) reversibly prolongs the duration of long-term potentiation (LTP) of the population EPSP amplitude in the CA3 subfield of the rat hippocampal slice preparation (Hopkins and Johnston, Neurosci. Abs. 8: 740, 1982). The experiments described here suggest that NE need only be present in the bath during the high-frequency train used to induce LTP to exert this action, and that the effect is mediated by B-adrenergic receptors.

Rat hippocampal slices were maintained in vitro at 32° C using standard techniques. Field potentials were recorded from stratum lucidum and pyramidale of the CA3 subfield in response to stimulation of the mossy fibers. LTP was typically induced with 100 Hz, 2 sec trains of 0.05 msec constant-current pulses. The amplitude of the population EPSP, expressed as a percentage of the pre-tetanic response, was measured as a function of time following the high-frequency train. Some slices were capable of demonstrating several similar LTP episodes in which the population EPSP amplitude decayed to baseline levels within 80 minutes. The time at which the population EPSP decayed to its half-maximal amplitude with respect to baseline (half-decay time) was used to quantify LTP duration. Half-decay times greater than 15 minutes and leftward shifts in I/O curves at 15 minutes post-tetanus were taken as evidence of LTP.

When NE was present only during tetanic stimulation, the mean± s.e.m. half-decay time was 105.6±7.1 minutes. This is similar to the value obtained in previous experiments in which NE was present during both tetanic stimulation and the decay phase (105.8±10.7 minutes). Similar measures for the control and wash episodes were 30.9±7.8 and 25.7±0.7 minutes, respectively.

The B-adrenergic agonist isoproterenol (1 uM) mimics the effect of NE on LTP duration. The mean half-decay time for experiments in which isoproterenol was present only during high-frequency stimulation was 112.5±3.6 minutes. The control and wash mean half-decay times were 23.9±5.9 and 25.1±0.3 minutes, respectively. Propranolol (10-100 nM), a B-receptor antagonist, blocks the NE effect on LTP, and reversibly blocks LTP. In contrast, the α-receptor antagonist yohimbine (1 uM) did not affect the action of 10 uM NE on LTP duration.

The mossy fiber synapses are electrotonically close to the cell bodies of CA3 pyramidal neurons (Johnston and Brown, J. Neurophys. in press). It may therefore be possible to explore the cellular basis of these effects using other electrophysiological techniques. (Supported by the McKnight Foundation and NIH Grants NS11535, NS15772, and NS18295).

248.PO **RAPID PROCESS FORMATION BY DISSOCIATED ADRENAL CHROMAFFIN CELLS IMPLANTED INTO THE RAT BRAIN.** U. Patel, W.J. Freed, R.J. Wyatt. Preclinical Neurosciences Section, Adult Psychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032

Mechanical dissociation of adrenal medullary tissue is a quick method for obtaining viable adrenal chromaffin cells for injection into discrete regions of rat brain (for method see Wells, M.R. and Patel, U., this volume). To study the properties of these cells after implantation, mechanically dissociated adult adrenal chromaffin cells were injected into the denervated striatum of rats with unilateral substantia nigra lesions. In some experiments, cells were incubated for one hour in 0.05 uCi/ml [^{3}H] Lysine for short term protein labeling before injection. Injected animals were processed for glyoxylic acid-induced catecholamine histofluorescence and autoradiography from one hour to 30 days after injection. Control animals were injected with heat-killed dissociated [^{3}H] Lysine labeled cells. After implantation some injected cells began to enlarge and form thin cytoplasmic processes within the first hour. Most of the injected cells had formed processes exceeding the diameter of the cell by four hours after injection. The formation of processes continued for the first 24 hours. After four days, implanted cells as identified by histofluorescence and autoradiography were found 2-5 mm away from the injection site. The cells appeared to have migrated primarily, but not exclusively, adjacent to blood vessels. The formation of processes by these cells was much more extensive than that which has been observed in adrenal medullary tissue implants into cerebral ventricles (Freed et al., Nature, 292:351, 1981). While the results indicate that some dissociated cells injected into the brain demonstrate different properties than whole tissue implants, the difference could be due to the more intimate contact between dissociated cell grafts and the host brain. The possibility of injecting dissociated cells with minimal damage to the host brain may increase the effects of these grafts and may facilitate transplantation of catecholaminergic tissue into higher vertebrate brains. Possible influences of such implants on the host brain are under study.

MUSCLE AFFERENTS

249.1 NON-SELECTIVE MOTOR INNERVATION OF BAG$_1$ INTRAFUSAL FIBERS IN CAT MUSCLE SPINDLES. J. Kucera, Dpt. of Neurology, Boston Univ. Sch. of Med., Boston, MA 02118

Studies of living cat muscle spindles and experiments using the glycogen-depletion technique have shown that dynamic γ or β motor axons supply nuclear bag$_1$ intrafusal muscle fibers and static axons supply nuclear bag$_2$ and chain fibers. However, it has been unclear whether some motor axons may co-innervate the bag$_1$ fiber together with other types of intrafusal fiber in the same spindle. To answer this question we have reconstructed the motor nerve supply to 10 whole and 6 half spindles from serial semithin (1 um thick) and periodic ultrathin transverse sections of tenuissimus muscles removed from anaesthetized cats (sodium pentobarbital 35 mg/kg) and stained with toluidine blue or uranyl acetate. The motor axons were traced from their origin in intramuscular nerve trunks to their intrafusal terminals.

Of the 28 poles of the bag$_1$ intrafusal fiber encountered, 23 received motor nerve supply and 5 did not. All the innervated bag$_1$ fiber poles received 1 to 3 (mean 1.4) selective motor axons that supplied this fiber type only. Four of the poles (17%) received, in addition, terminals from a non-selective motor axon that also innervated one, but not more, of the chain fibers in the spindle. No motor axon branched to supply both bag$_1$ and bag$_2$ fibers. The chain fibers co-innervated with bag$_1$ fibers tended to have a higher density of nuclei at the equator, a greater polar length and a less granular appearance when stained with toluidine blue than most other chain fibers. Terminals of the non-selective motor axons on the co-innervated bag$_1$ and chain fibers were morphologically and ultrastructurally dissimilar. It is suggested that instances of innervation of the (dynamic) bag$_1$ fiber in common with a (? static) chain fiber represent an integral and, presumably, functionally meaningful part of the motor pattern in some cat spindles.

249.2 RELATION OF SINGLE FIBER EPSP AMPLITUDE IN SPINAL MOTONEURONS TO LOCATION OF AFFERENT ENTRY. J.E. Zengel, G.W. Sypert and J.B. Munson. VA Med. Ctr. and Depts. of Neuroscience and Neurological Surgery, Univ. of Fla. Coll. of Med., Gainesville, FL, 32610.

Many factors may be involved in determining the amplitude of EPSPs in spinal motoneurons. One factor that may affect the amplitude of EPSPs generated by muscle spindle afferents is the location of the motoneuron relative to the spinal cord entry point of the afferent. The present study investigated the amplitude of EPSPs generated in motoneurons by single homonymous muscle spindle group II afferents as a function of the location of the motoneuron relative to the rostral-caudal entry location of the afferent.

Three to 4 mm of spinal cord in the region of the L7-S1 dorsal root junction were systematically explored at 100 µm intervals in the longitudinal direction to locate medial gastrocnemius (MG) motoneurons. The spike-triggered averaging technique was used to record single-fiber EPSPs generated in these motoneurons by single spindle group II afferents. EPSP amplitude (or failure) was related to the entry level of the triggering afferent, which was determined at the conclusion of the experiment.

We measured the amplitude of 81 single fiber EPSPs generated by 4 spindle group II afferents (104 afferent-motoneuron combinations). We found that afferents entered the spinal cord at locations which were rich in MG motoneurons as indicated by the ease of recording MG motoneurons at these locations. Afferent-to-motoneuron connectivity and single fiber EPSP amplitude were greatest in the region of afferent entry. On average, connectivity and amplitude declined progressively at more rostral locations, but each again increased in magnitude at least over the interval 1.0-1.5 mm rostral of the afferent entry location.

These data, along with consideration of other anatomical and electrophysiological data (Ref. 1,2), suggest that EPSP amplitude and connectivity are each greatest at locations where afferent collaterals enter the motoneuron nucleus. A second region of relatively powerful innervation (high EPSP amplitude and connectivity) appears to exist 1.0-1.5 mm rostral to afferent entry, where another collateral probably descends into the motoneuron nucleus (Ref. 2). Additional such regions probably exist further rostrally.

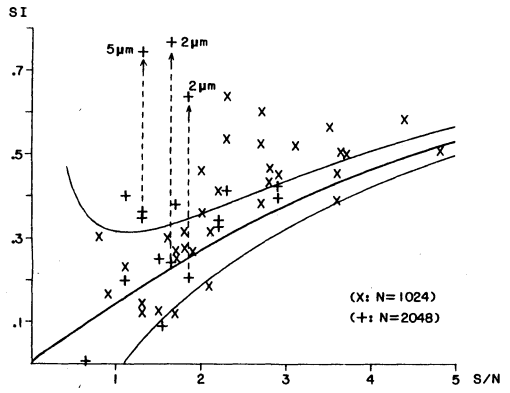
References: 1. Munson, Fleshman and Sypert, J. Neurophys. 44, 713-725, 1980. 2. Brown, Organization in the Spinal Cord. Berlin: Springer-Verlag, 1981.

Supported by the MRS and RERDS of the Veterans Administration and NINCDS (NS 15913).

249.3 THE PRESENCE OF AFFERENT SYNCHRONY IN A PASSIVE MUSCLE AT FIXED LENGTH. T. M. Hamm, D. D. Roscoe, R. M. Reinking* and D. G. Stuart. Department of Physiology, University of Arizona Health Sciences Center, Tucson, AZ 85724.

We have previously reported the use of an index (SI) which tests for synchrony between neuronal spike trains by comparison of rectified and non-rectified averages, triggered by a reference train (Roscoe et al, Adv. Physiol. Sci. 1: 219-228, 1980; Reinking et al, Soc. Neurosci. Abstr. 7: 558, 1981). This index has now been modified to increase its sensitivity. Contrary to our previous findings, we now report the presence of afferent synchrony emanating from the passive, de-efferented cat medial gastrocnemius muscle when held at fixed length (see figure). This synchrony is subtle, as judged by comparison of SI values obtained at fixed muscle length and during small (2-5 μm) "quick" stretches. The significance of these results for interpretation of spike-triggered averages will be discussed.

Supported by USPHS grants NS 07888, NS 17887 and HL 07249.
Present address of D. D. Roscoe: Dept. of Surgical Research, St. Luke's Hospital, Cleveland, OH 44104.



SI values (based on averages of N = 1024 and 2048) plotted as a function of signal-to-noise ratio (S/N) of the reference-afferent spike in the neurogram. Solid lines show the expected value ± 2 S. D. (at N = 1024) for the condition of complete asynchrony. Dashed arrows show increase in SI with application of quick (5 ms duration) stretches of indicated amplitude.

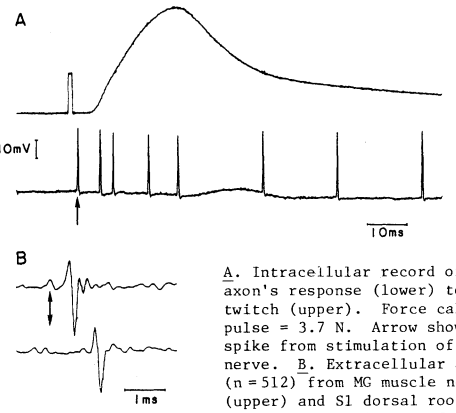
249.4 SELECTIVE ACTIVATION OF SINGLE TYPE-IDENTIFIED MUSCLE RECEPTOR AXONS. C.-S. Yuan*, T. M. Hamm, R. M. Reinking* and D.G. Stuart. Department of Physiology, University of Arizona Health Sciences Center, Tucson, AZ 85724.

For the unambiguous study of Ib-motoneuronal connections, we have adapted the technique of Zealear and Crandall (J. Neurosci. Methods 5: 47-54, 1982) for stimulating single motor-unit axons to stimulating and recording from single Ib axons within a muscle nerve.

A stepping motor is used to advance a glass microelectrode into a fascicle from the cat medial gastrocnemius (MG) muscle within the sciatic nerve, which is mounted on a stable platform. Following penetration of an axon or its myelin sheath, the axon can be identified using conventional tests and its continuity with the spinal cord and the ability to activate the axon selectively verified by extracellular dorsal-root and muscle-nerve recordings, respectively (see figure).

This technique provides an alternative to the study of muscle receptor-motoneuronal connections using stimulation of single afferents in dorsal-root filaments (Honig et al, J. Neurophysiol. 49: 886-901, 1983) or a dorsal-root ganglion (Willis et al, Brain Res. 9: 152-155, 1968).

Supported by USPHS grants NS 17887, NS 07888 and HL 07249.



A. Intracellular record of a Ib axon's response (lower) to MG twitch (upper). Force calibration pulse = 3.7 N. Arrow shows direct spike from stimulation of muscle nerve. B. Extracellular averages (n = 512) from MG muscle nerve (upper) and S1 dorsal root (lower) following stimulation of Ib axon (at arrows).

249.5 PATTERNS OF DISCHARGE OF PRIMARY AFFERENTS AFTER ADMINISTRATION OF 4-AMINOPYRIDINE IN THE CAT. R. Dubuc, J. Provencher* and S. Rossignol. Centre de recherche en sciences neurologiques, Département de physiologie, Université de Montréal, Montréal, Québec, Canada H3C 3J7.

Rhythmic bursts of activity (3-7Hz) are recorded from peripheral nerves following the injection of 4-aminopyridine (4-AP) in the decerebrate and paralyzed cat (Rossignol, S. et al., Soc. Neurosci. Abstr., 7, p. 863, 1981). Similar discharges are also recorded from the proximal stump of cut dorsal or ventral roots (Dubuc, R. et al., Soc. Neurosci. Abstr., 8, p. 957, 1982). Therefore it appears that the rhythmic discharges recorded from a mixed nerve in the periphery is a composite of both ventral root activity and antidromically conducted discharges in the primary afferents. In the present study primary afferent units were recorded in the dorsal roots.

Cats were decerebrated at the precollicular level, paralyzed and artificially ventilated. A spinal transection was effected at Th 13 and the lumbar enlargement was exposed. Glass-coated tungsten electrodes were utilized to record single units from the exposed dorsal roots. Cuff or Ag/AgCl hook electrodes were used to record and/or stimulate peripheral nerves which were cut distally to the electrodes. The conduction velocity of the units was determined from the latency of the stimulation-evoked discharge and the distance between the recording and stimulating sites measured post-mortem.

A total of 133 primary afferent units were recorded from eleven cats in which rhythmic discharges were observed from peripheral nerves after an i.v. injection of 10 mg/kg of 4-AP. 51% of the units discharged in bursts of spikes (200-400 Hz) which in some cases had the same rhythmicity as that observed in the peripheral nerves. Another 26% of primary afferents showed a tonic discharge pattern with a rigourously constant interspike interval. The rate of discharge of those cells ranged between 10 and 40 Hz. Finally, the remaining 23% of the cells were either silent or had an irregular single spike pattern of discharge. It was found that the conduction velocity of the great majority of bursting cells ranged between 40-80 $m \cdot s^{-1}$ while that for the majority of the tonic cells was between 80 and 120 $m \cdot s^{-1}$.

It is concluded that 4-AP may exert differential effects on primary afferents of different size. Experiments are underway to determine whether such differential effects are related to different types of primary afferents.

(Supported by a Group grant of the Canadian MRC and a studentship from FCAC to R. Dubuc).

249.6 ANATOMICAL CORRELATES OF MOTOR UNIT/TENDON ORGAN (MU/TO) RESPONSE PATTERNS. J. M. Spielmann* and E. K. Stauffer. Department of Physiology, Sch. of Med., Univ. of Minnesota, Duluth, MN 55812.

An inherent characteristic of MU/TO physiology is that the Ib afferent responds to the intramuscular force coupled to the receptor's capsule rather than to the total force directed along the common tendon of the whole muscle. Analysis of MU/TO responsiveness is hampered, however, by the lack of data from the same preparation to make a direct comparison of physiology and anatomy. The present investigation was designed to examine the functional arrangement of single identified TOs, single identified MUs, and to relate this structure to the Ib afferent signal.

Soleus Ib afferents were isolated from subdivided ventral rootlets and their associated TOs were located on the posterior aponeurotic surface by finding the point of greatest Ib response to external pressure applied with a fine-tipped probe. Single MUs from subdivided ventral rootlets were studied if their contraction elicited a response from the TO. TO firing patterns were recorded during whole muscle and MU twitches. MUs were glycogen-depleted with intermittent trains (40 Hz) of short tetani (330 msec on, 670 msec off) for 1½-2 hr. after which the muscle was removed, trimmed, and frozen in isopentane (-160°C). Serial sections (10 μ) were stained separately: (1) with periodic acid Schiff to track the depleted MU fibers (DF); and (2) modified Gamori trichrome to examine the MU/TO anatomy.

All TOs that gave the typical accelerated discharge during the rising phase of whole muscle and/or MU twitch force profile had a DF attached specifically in series to the proximal tip (collar) of the end organ. In addition, TOs with an in series DF usually had a discharge pattern that extended throughout almost the entire duration of the MU twitch profile. Deviations from this common response could be explained in terms of an assumed unloading effect of nearby in parallel DFs from the same MU. In one case, a second DF attached onto the TO capsule near the equatorial region of the receptor. In this instance, the number of Ib afferent spikes was greatly reduced and the time of their occurrence was limited to the initial portion of the twitch force. We found no relationship between the TO discharge and the location of the inserting DF amongst the other circumjacent inserting fibers. There was, however, a direct relationship between the size of the TO's receptive field (i.e. total number of muscle fibers that inserted into the collar) and the magnitude of its Ib afferent discharge. These findings are consistent with and confirm earlier interpretations of MU/TO response patterns which rested solely on either physiological or anatomical data from complimentary but separate studies.

Supported by Univ. Minn. Grad. School and Minn. Med. Found.

249.7 ULTRASTRUCTURAL LOCALIZATION OF CARBONIC ANHYDRASE ACTIVITY IN RAT SENSORY SPINAL NEURONS, AXONS, AND MUSCLE SPINDLE SENSORY AND MOTOR TERMINALS. D.A. Riley, S. Ellis*, and J.L.W. Bain*. Med. College Wis., Dept. of Anatomy, Milwaukee, WI 53226 and NASA Ames Research Center, Moffett Field, CA 94035.

Recently, we demonstrated histochemically that a subset of myelinated axons in rat peripheral nerves exhibited high levels of carbonic anhydrase activity (Riley et.al., 1982, J.H.C. 30:1275). The majority of the reactive axons were sensory. The present study examined the subcellular distribution of carbonic anhydrase activity in lumbar (L4,5) dorsal and ventral root axons, spinal ganglion cells, and the sensory and motor nerve endings of muscle spindles in extensor digitorum longus muscles. Male Sprague-Dawley rats (325-350 g) were perfused with 2.5% glutaraldehyde. Tissues were sectioned (20 μm) with a vibratome and incubated on gelatin-coated plastic slides in Hansson's medium. Following osmication at pH 6 in osmium-ferricyanide, sections were embedded in Epon. Specificity of staining was confirmed by inhibition with 10^{-5} M acetazolamide. In the spinal roots examined, nonmyelinated axons were always unstained. The axoplasm of highly reactive myelinated axons was densely stained throughout except for the cisternae of smooth endoplasmic reticulum. The staining in moderately reactive axons and in sensory neurons was predominantly localized in discrete patches on the cytoplasmic surface of the endoplasmic reticulum. In gamma motor terminals, similar patches of staining were present on synaptic vesicles and the endoplasmic reticulum. The cytoplasm of primary sensory terminals (Ia) was densely stained throughout. The intracrista spaces of mitochondria in reactive somata, axons and terminals were stained. The cytoplasmic distribution of carbonic anhydrase indicates that it is not secreted by neurons. The enzyme may regulate intracellular pH and CO_2 levels during impulse conduction. The preferential deposition on the endoplasmic reticulum indicates a functional association with this organelle. The present results identified a portion of the reactive sensory neurons as primary spindle afferents and demonstrated that some of the reactive axons in the ventral roots were gamma efferents. These findings are consistent with our hypothesis that carbonic anhydrase is highest in tonically-active cells. This work was supported by the Institutional Research Committee of the Medical College of Wisconsin and a NASA Ames University Consortium NCA-OR665-102.

249.8 MUSCLE SPINDLE DISCHARGE DURING SCRATCH REFLEX OF THE SPINALIZED TURTLE. Z. Hasan. Department of Physiology, University of Arizona, Tucson, AZ 85724.

In the study of muscle receptors and movement, the scratch reflex of spinalized vertebrates offers a potential simplification in that only one limb participates in the movement. The low-spinal turtle is of special advantage since in this animal the programs for scratching can be elicited readily, and also because sufficient information already exists regarding the muscular synergies and the kinematics.

Turtles (Pseudemys) were decerebrated under ether, ventilated artificially, and spinalized at the D2 level. Part of the carapace was removed, and the loss of the dorsal anchoring of the ilium was compensated. Unit afferent activity was recorded, using a monopolar electrode (75μm S.S.), from fine filaments, 1-2mm long, of the otherwise intact S1 dorsal root. Denervation of the shank and the foot left intact the contribution to the S1 root from knee flexor and/or hip retractor muscles with origins in the pelvic girdle.

Attention was focused on the Flexor Tibialis Internus (FTI) and Iliofibularis (IF) muscles. Electromyograms (EMGs) were recorded from these adjacent, two-joint muscles. Lack of cross-talk was confirmed since, in the A1 phase of the rostral scratch, IF was active while FTI was not, as has been reported. However, in the caudal scratch, the two muscles acted more synergistically, although IF activity always commenced before that of FTI. A length-gauge which relied on the resistance of saline in a Silastic tubing (0.9mm OD), was implanted in parallel with IF.

Spindle afferents were identified by the characteristic pause in discharge that occurred following an electrical stimulus to the muscle while the shank was held rigidly. During scratch-related muscle contraction, these afferents exhibited the full range of behavior from cessation of discharge to increase of the discharge rate during shortening. However, a particular afferent, if it increased its discharge rate during contraction of the receptor-bearing muscle, did so only after a particular level of EMG activity appeared to have been attained, and the increase was greater when the EMG was higher in amplitude. Thus, more vigorous cycles were associated with greater afferent discharge during active shortening. However, the peak rate of discharge was no higher than 40 imp/s.

These observations are consistent with spindle innervation by branches of skeletomotor axons in turtles. Mammalian spindle afferents, in contrast, have not been reported to exhibit silence at the onset of contraction in active movements. This difference may underlie the significance of the gamma system.

249.9 ORIGINS OF SENSORY FIBERS OF THE MASTICATORY AND EXTRAOCULAR MUSCLES IN THE RAT. H.Y. Bai* Z. Ye* and W.H.A. Yu (SPON: A.N. Bender). Department of Anatomy, Mount Sinai School of Medicine, New York, N.Y. 10029.

The localization of the somata and sensory neurons innervating muscles of mastications and the extrinsic eye muscles were investigated by means of retrograde transport of horseradish peroxidase (HRP) which was injected intramuscularly (5-10 ul of 10% solution) or applied directly to the proximal stump of the transected nerves supplying the corresponding muscles. The HRP tracing method revealed that the jaw-closing (temporalis, masseter, and medial and lateral pterygoid) muscles and the extraocular muscles received afferent fibers from neurons located in the semilunar ganglion (SG) and in the mesencephalic trigeminal nucleus (MTN). The jaw-opening (mylohyoid and anterior belly of digastric) muscles, on the other hand, did not receive afferents from the MTN. Projections to the masticatory and extraocular muscles from the SG and the MTN were unilateral except those to the muscles innervated by the oculomotor nerve, which received a bilateral innervation from the MTN. HRP labeled neurons in the MTN exended from the pons at the level of the nucleus of the abducens nerve to the midbrain rostral to the superior colliculus. There was a topographic organization in the MTN in that the neurons innervating jaw-closing muscles were represented caudally, while those supplying extraocular muscles occupied a more rostral level. Following each HRP administration a large proportion of the motoneurons was also labeled only in the appropriate motor nucleus, which served to indicate the reliability and precision of the delivery of the tracer. The abducens and trigeminal motoneurons were labeled unilaterally. The oculomotor and trochlear motoneurons were labeled bilaterally suggesting the presence of crossed and uncrossed fibers. In conclusion, the jaw-closing and extraocular muscles of the rat received afferent fibers from the semilunar ganglion and the mesencephalic trigeminal nucleus. The dual origins may represent functional segregation, each subserving different sensory modality.

250.1 SENSITIVITY OF NEURONS IN VESTIBULAR NUCLEUS TO ACTIVE AND PASSIVE HEAD MOVEMENTS IN ALERT RHESUS MONKEY. Sat Bir Khalsa*, D.W.F. Schwarz, R.D. Tomlinson*, & J.P. Landolt, Lab. of Otoneurology, Univ. of Toronto, Toronto, Ont., Canada, M5S 1A8.

Recent electrophysiological studies in the squirrel monkey (Goldberg, J.M. & Fernandez, C., 1980, J. Neurophysiol., 43: 986-1025) have indicated that the efferent vestibular system is capable of modifying primary afferent vestibular information. If the efferent vestibular system modifies the afferent signal in connection with a motor program for active head movements, then this should be apparent as a difference in neuronal firing characteristics recorded at the level of the vestibular nucleus. Rhesus monkeys were trained to fix a small target, which was used to evoke head movements. Crowns were implanted to allow for the mechanical fixing of the animal's head, for the recording of the EOG via silver wire electrodes, and for the recording of vestibular neurons in the vestibular nucleus through a recording chamber.

Tungsten micro-electrodes were used to isolate single units, and each unit was recorded under the following conditions: a) the animal's head fixed to a table oscillating in the horizontal plane with an earth fixed target, b) the animal's head fixed to the table oscillating in the horizontal plane with the target fixed on the table (a VOR cancellation task), c) the animal's head fixed to a stationary table with a moving target, d) the animal's head free to move in the horizontal plane within an angle of 90° left to right in order to pursue a moving target with both head and eye movements. Neuron spikes were transformed into digital pulses and collected into 5 ms bins and the head, eye and table movements were recorded.

The sensitivity of the units to horizontal vestibular stimulation in the above conditions was measured by calculating the peak velocities and corresponding spike rate of modulations. These values were assembled and a regression curve plotted to represent the characteristic sensitivity of each unit in the various conditions. Our preliminary data suggest that there is no difference between horizontal unit sensitivity in active head movements as compared with passive head movements under the conditions of this experiment. It is therefore unlikely that the efferent vestibular system contributes sensitivity changes due to head rotation motor programs, at least in the range of head velocities used.

Supported by MRC and DCIEM Research Contract 327-9016/8FU79-00252.

250.2 DIFFERENCES IN COMPENSATORY EYE MOVEMENTS DURING ACTIVE AND PASSIVE HEAD MOVEMENTS IN PRISM-ADAPTED CATS. R.M. Douglas & D. Guitton. AMRU and MNI, McGill Univ., Montreal, Canada H3G 1Y6

In the normal cat, gaze shifts are accomplished with a combined eye saccade and rapid head movement, while between saccades, a near constant gaze direction is maintained by the VOR. However, when the world is viewed through reversing prisms, this pattern is no longer appropriate. Ideally the saccade and head movement should go in opposite directions, but this was not seen in the present experiments. The most striking consequence of prolonged reversed vision was that the compensatory eye movement in the active state did not assume a uniformly low gain.

Three cats wore left-right reversing prisms for 2-12 weeks, during which time they were required to make orienting eye-head movements to obtain food, and to walk and jump around the laboratory. As expected from previous studies, the passive VOR gain, measured during 0.1-1.0 Hz sinusoidal rotation in the dark, dropped to about 0.1-0.3 during the first week and declined only slightly thereafter. In contrast, ocular gain was not consistently low when the adapted cats made voluntary active eye-head movements. Immediately after each saccade there was a brief period (<100 ms) during which the gain often reached surprisingly high values (0.8-0.9). For a wide range of head velocities and amplitudes, the mean gain from the end of the saccade to the end of the head movement was about 0.6. During passive rotation, there was no similar transient increase in gain after quick phases. This difference between active and passive gain measurements was not due to involvement of neck afferents, as the gain was always very low when the experimenter rapidly moved the cat's head while keeping the body stationary. These observations show that the pattern of vestibular activity was not critical, but rather suggest a role for the central motor program. This was corroborated by observations on eye-head movements evoked by electrical stimulation of the superior colliculus. During evoked eye-head movements the gain increased gradually from near 0.0 to 0.9, and this did not depend on when the evoked saccades occurred during the head movement. Thus the appearance of high gain after saccades during voluntary eye-head movements was not causal, but was more closely linked to the head movement component.

The utility of the brief high gain periods is suggested by the fact that in both the normal and adapted state, the combined movements began and finished with the eyes a few degrees from center. Without the high gain period the saccades, which even in the adapted cat always occurred with every head movement, would have left the eyes eccentric in the orbit and thus poorly positioned for the next movement.

250.3 PHYSIOLOGICAL CHARACTERIZATION OF NECK MUSCLES ACTION IN GAZE SHIFTS. A. Roucoux, M. Crommelinck::, A. Al-Ansari:: and C. Veraart::::. Lab. of Neurophysiology, Univ. of Louvain, Fac. of Med., B-1200 Brussels, Belgium.

Neck muscles play a major role in gaze displacement and stabilization. Their anatomical organization reflects, in its complexity, the numerous degrees of freedom of the head. In this study, we have reduced the number of these degrees of freedom to two on the basis of a number of observation demonstrating a close correlation between eye movement and neck muscle activity. More specifically, our starting point was our observation that, in the head fixed alert cat, a horizontal eye position signal is continuously sent to some of these muscles. The activity of a dozen of neck muscle pairs, most of them dorsal, has been recorded in alert cats, with head fixed or free, together with gaze horizontal and vertical position. Eye movements and, when pertinent, head movements, were recorded by the magnetic field technique. In a first series of experiments, with head fixed, the neck E.M.G. was correlated with both horizontal and vertical components of eye displacement. Most of the investigated muscles, to a variable extent, exhibited an increase of their discharge level when the eye was displaced towards the side of the muscle or upward. The muscles have been classified on anatomical grounds as horizontal or vertical movers of the head. Physiologically they can be distinguished by their "preferred direction" – i.e. the direction of the eye movement yielding the maximum discharge –. A muscle like the biventer cervicis, thought from the anatomy of its insertions to elevate the head, is also modulated by horizontal eye displacements. Its preferred direction, however, tends to be more vertical than that of the splenius, known as a horizontal mover. In some instances, motor units could be isolated. They had, in a given muscle, different thresholds and showed successive recruitement with increasing eye eccentricity. The same muscles were also recorded in the head free, body restrained, condition. As a whole, the different muscles acted according to their "preferred direction", as determined with head fixed. None of the muscles was exclusively horizontal or vertical.

The hypothesis is made that these muscles have a physiological pulling direction which is oblique and that pure horizontal or vertical gaze displacements, performed with the help of the head, require a tight and rather complex coordination of them. These results also raise the question of the mechanisms responsible for the distribution of gaze motor orders to sets of muscles having such different pulling planes as those moving the eye and the head.

:::: Research Associate, N.F.S.R. Belgium.

250.4 LATERAL CUNEATE NUCLEUS: VESTIBULAR RESPONSES IN NECK AND SHOULDER AREAS BUT NOT DISTAL FORELIMB. David W. Jensen, Dept. Otorhinolaryngology and Program in Neuroscience, Baylor College of Medicine, Houston, Texas 77030.

The lateral cuneate nucleus (LCN) in the dorsolateral caudal medulla relays somatosensory input from ipsilateral C1 - T2 to the cerebellum. Vestibular nerve fibers also course through regions of the nucleus where unitary responses to electrical stimulation of the vestibular nerve are recordable (Jensen and Thompson, ARO Mid-Winter Meeting, 1980). The purpose of this study was to determine the location of the somatosensory receptive fields of these vestibular-responsive LCN regions. Guinea pigs were used, and were anesthetized with nembutal, fentanyl and droperidol. Electrical stimuli were delivered to the vestibular nerve in intensities relative to the threshold of the vestibular nuclear N1 potential while a glass micropipette was advanced through the ipsilateral LCN. Somatosensory stimuli were applied to the body surface with a blunt probe.

Vestibular-responsive units were largely confined to the rostral third and a ventral strip region of the mid rostrocaudal third of the nucleus. As seen with an extracellular microelectrode, these units showed slowly adapting responses to deep pressure applied to the ipsilateral neck or shoulder surfaces. Not all neck and shoulder units were vestibular-responsive, however. In addition, vestibular stimulation never activated a unit that had a receptive field on the distal aspect of the ipsilateral forelimb.

From these results it is speculated that postural control mechanisms may involve a selective vestibular sculpturing of somatosensory input that is most reflective of the body's center of gravity.

Supported by a grant from the N.I.H.

250.5 TWO-COMPONENT HUMAN OKAN. R.M. Jell, D.J. Ireland* and O. Proden*
Physiology and Otolaryngology, University of Manitoba, Winnipeg, Canada, R3E OW3.

The time course of optokinetic afternystagmus (OKAN) in animals and in humans has previously been characterized by a single time constant (one-component) decay after lights out, presumably related to some form of neuronal storage mechanism. Close examination of many OKAN records from normal humans leads us to conclude that this model is inadequate. Our results support a two-component model with terms representing an initial rapid decay followed by a slow decay.

OKAN was produced by full field stimulation in a rotating (40 deg/sec) vertical drum 80 cm in diameter in which the subject sat. Black vertical stripes (visual angle 2 deg) alternated with white bands (18 deg) on the inner surface of the drum. Chart records obtained by D.C. electrooculography were digitized by graphics tablet. Slow phase velocities after lights out were plotted against time and coefficients of the equation: SPV=A. exp (-Bt) + C.exp (-Dt) were computed by non-linear regression analysis.

In 21 subjects tested, the rapid decay was found to have a mean time constant of 0.93 secs (SD 0.83 secs) and initial value 39.0 deg/sec (SD 27.7 deg/sec) while the corresponding slow decay values were 51.4 secs (SD 55.2 secs) and 7.4 deg/sec (SD 3.7 deg/sec) respectively. The differences between rapid and slow decay parameters were significant at $p < 0.005$.

These data support the conclusion that OKAN decay is a two-component process, and that two different storage mechanisms are involved in normal human OKAN. Both storage mechanisms are presumably charged by the optokinetic stimulus during the lights-on period, but differing initial values obtained in the regression analysis suggest that the gains of the two discharge pathways are different. A suitable model would consist of two separate integrators, each with different forward and feedback gain factors, acting in parallel such that their outputs summated to generate a net eye drive signal to the motor nuclei of the extrinsic eye muscles.

Supported by the Medical Research Council of Canada and the Manitoba Health Research Council.

250.6 SACCADE-LOCKED INITIAL STEP IN OKAN DECAY. D.J. Ireland*, R.M. Jell and O. Proden*. (SPON: K.W. Cheng). Physiology and Otolaryngology, University of Manitoba, Winnipeg, Canada. R3E OW3.

With appropriate stimulus conditions, optokinetic nystagmus is immediately followed in the dark by a time-dependent decay of slow phase velocity. This decay is called optokinetic after-nystagmus (OKAN). In normal animals and man, OKAN has been shown to be preceded by a step decrease in SPV to an initial value for the decay. The size of the step is dependent upon the velocity of the optokinetic stimulus. Our OKAN records reveal, in about 20% of cases, a step discontinuity in an early slow phase which is time-locked to the preceding saccade. This discontinuity usually occurs within one slow phase of the time of lights-out, and may occur whether lights-out occurs during a slow phase or during a saccade.

Analysis by graphics tablet and digital computer of 68 clearly visible discontinuities which we call "breaks", obtained in normal humans by full field stimulation, revealed that at the break the slow phase velocity (SPV) suddenly dropped by a mean of 48%. Pre- and post-break SPVs were of constant velocity, with a mean pre-break SPV of 42.3 deg/sec (SD 23.94) with stimulus velocity of 40 deg/sec. Mean time from the end of the previous saccade to the break was 0.248 sec (SD 0.116). These values were independent of the point in time at which lights-out occurred.

It would appear that the onset of the discharge process is time locked to the end of the prior saccade, following it with a delay of approximately 250 msec, and that at this point, the step decrease in velocity occurs before OKAN commences. In about 80% of records examined, no step could be seen by visual examination, suggesting that other unknown factors may cover up or prevent the occurrence of the discontinuity.

Supported by the Winnipeg Health Sciences Centre Research Foundation and the Medical Research Council of Canada.

250.7 SIGNALS USED TO MAINTAIN SMOOTH PURSUIT EYE MOVEMENTS IN MONKEYS: EFFECTS OF SMALL RETINAL POSITION AND VELOCITY ERRORS. E. J. Morris* and S. G. Lisberger (SPON: M. I. Law). Dept. Physiol., Div. Neurobiol., UCSF, San Francisco, CA 94143

The aim of our study was to determine the signals used by the monkey pursuit system to maintain accurate tracking of smoothly moving visual targets, and particularly to study the role of small retinal position and velocity errors. Monkeys tracked a moving spot that appeared on a screen when they pressed a bar; after an unpredictable interval (500-2400 ms) the spot dimmed and monkeys were rewarded if they released the bar within 600 ms. Eye position was monitored by the magnetic search-coil technique.

Each experimental trial began with the monkey tracking a target moving horizontally at 15°/s. To allow precise control of visual inputs, we then opened the visual feedback loop by driving target position with a signal composed of the eye position monitor plus any desired error. When target position was stabilized on the fovea (imposing zero position and velocity errors), pursuit was maintained at the initial velocity. An objective technique was used to assess technical errors in opening the loop and to verify that those errors were too small to account for the maintenance of pursuit. Therefore visual errors are not necessary to maintain pursuit.

During maintained open-loop pursuit at 15°/s across a diffusely-illuminated background, imposition of small velocity or position errors caused large changes in smooth-pursuit eye velocity at latencies of 100 ms or less. For velocity errors up to 1.5°/s, eye acceleration increased monotonically up to 50°/s^2. For position errors up to 0.7°, accelerations increased up to 30°/s^2; for larger errors (up to 1.5°), the acceleration response saturated or decreased. If position and velocity errors were oppositely directed, they opposed one another in their effect on eye velocity. When the background was dark, the response to position errors was almost eliminated, but velocity errors were still effective, and eye velocity was still maintained in the absence of errors. For initiation of pursuit (eye starting from rest), small velocity errors caused accelerations similar to those produced during maintenance at 15°/s but position errors were much less effective: monkeys attempted to correct them using mainly saccades.

Our data demonstrate that at least three signals can be used to maintain accurate pursuit: 1) retinal velocity error, 2) retinal position error, and 3) a form of velocity storage (consistent with theories of positive feedback through the flocculus). The fact that position errors are utilized selectively during the maintenance of pursuit and in the presence of background illumination suggests that this error signal may come from a system that detects velocity contrast between target and background.

(Supported by EY03878 and the McKnight and Sloan Foundations.)

250.8 INTERMITTENT ILLUMINATION: A BETTER METHOD TO CHARACTERIZE HUMAN VELOCITY STORAGE DURING OPTOKINETIC STIMULATION? Stephen Liben* and Bernard Segal (SPON: A.Gonshor), Aviation Medical Research Unit Dept. Physiol., McGill Univ., Montreal, Quebec, Canada H3G 1Y6.

When man is suddenly exposed to constant velocity full-field optokinetic stimulation, slow-phase eye-velocity rapidly approaches stimulus velocity and rarely exhibits the gradual build-up (eg, (1)) seen in other species. Thus, in humans, it is not readily apparent that a hypothesized (1) slowly responding 'velocity storage' mechanism becomes gradually charged by such stimulation. However, subsequent to extinguishing all illumination, the presence of velocity storage is suggested by the persistence of a gradually declining component of optokinetic after-nystagmus (OKAN). Previously (1,2), the charging characteristics of velocity storage have been estimated from the relationship between the initial value of OKAN, and the duration of the preceding constant-velocity optokinetic stimulus (ie by many separate 'variable-duration illumination' experiments). We now suggest that these characteristics can be obtained using 'intermittently-illuminated' optokinetic stimuli, provided that darkness intervals are too brief to affect sluggishly responding velocity storage processes, yet are long enough to estimate the initial value of OKAN due to each darkness interval. The present study aims to compare the effectiveness of these two approaches. Methods: First, the variable-duration illumination experiments of Cohen et al (2) were essentially repeated. Next, intermittent illumination (light-on: 9.7s; light-off: 2s) was employed while subjects viewed a drum rotating with 0.01 Hz, 60°/s square wave angular velocity profiles for 3-6 min. Because the frequency of darkness intervals (0.085 Hz) was a non-integer multiple of the frequency of drum oscillation (0.01 Hz), darkness intervals occurred at different phases of drum rotation in successive cycles. Eye velocity at the end of all darkness intervals (initial OKAN estimates) was then suitably stimulus-locked averaged. Results: Modulation of velocity storage mechanisms obtained using intermittent-illumination was approximately exponential (time constant, T: 5-11s; saturation value, S: 9-17°/s), and agreed (generally T and S values were within +20% in the same subject) with that obtained by variable-duration illumination (T: 4-13s; S: 12-17°/s). Moreover, the intermittent-illumination results were usually obtained in 30-50% less time. Thus intermittent-illumination appears to be a better method for the characterization of velocity storage mechanisms because experiments are shorter and because systems analysis can be used more efficiently due to the potential to employ arbitrary stimulus velocity profiles.

References: 1: J. Physiol. 270: 321 (1977); 2: Ann.N.Y.Acad.Sci. 374: 421 (1981).

(Supported by Canadian Medical Research Council).

250.9 RAPID EFFECTS OF VISION REVERSAL ON HEAD-EYE COORDINATION. G. Melvill Jones, D. Guitton, A. Berthoz*° and M. Volle*. Aviat.Med. Res. Unit, and Montreal Neurol. Inst., McGill Univ., Montreal, Canada, °Lab. Physiologie Neurosensorielle, CNRS, Paris, France.

A previous long term study demonstrated the development of a variety of interesting new strategies in head-eye coordination, after about 3-weeks of maintained optical reversal of vision. (Berthoz & Melvill Jones, Euro.Neurosci.Assn. Abstr. 1981). The present study on 2 human subjects investigated the early development of these changes during the first 6 hrs of the reversed vision experience. Horizontal head-eye coordination was tested by active head movements either in the light or dark. The task was to maintain fixation on the reversed (moving) image of a single earth-fixed target during a rapid voluntary change of head position. An electromagnetic brake could unexpectedly prevent an intended head movement.

Control tests consistently yielded VOR gains close to unity with no eye movement after sudden head brake. During the 1st hr. of reversed vision the induced eye movement was typically composed of 3 main sequential phases. 1) A short period of VOR whose gain was markedly reduced within the 1st few trials. 2) A 'catch-up' saccade in the direction of reversed image movement (i.e. same direction as that of the head) whose latency was compatible with visual triggering. 3) A smooth eye movement which could either resemble a further reduced 'VOR', or occur in the reversed direction, sometimes reaching velocities similar to that of the head, as is required for following the reversed target image. Tests in the 6th hr. yielded similar but more developed patterns of adapted response, including a decrease of saccade latency towards synchrony with head movement onset. Prevention of intended head movement was usually followed in both 1st and 6th hr test series by a particularly interesting new smooth eye movement in the appropriate (reversed) direction which, being synchronous with the intended head movement, suggests a newly established 'motor program'. Head rotation in the dark usually produced leading eye movements resembling slowed saccades. All measured effects became progressively more marked from 1st-6th hr. trials.

The reduction of initial VOR suggests either very early adaptive attenuation of gain, or an effect due to a new mental set, since it was manifest on initiation of head movement and hence could not be due to vision. The post saccadic smooth eye movement (see 3 above) could have comprised a combination of 'visuomotor' response (latency > 120 ms) and the 'motor program' mentioned above. Interestingly, the post saccadic movement sometimes seemed to merge with the preceding 'catch-up' saccade to produce a 'glissade' type of movement remniscent of adaptive readjustment of the neurological pulse-step ratio.

Supported by Medical Research Council of Canada.

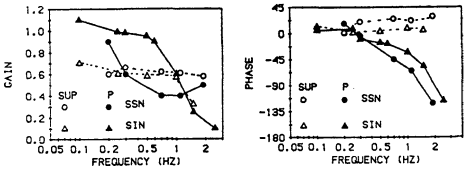
250.10 DOES PURSUIT CONTRIBUTE TO SUPPRESSION OF THE VOR? P. McKinley, B. Peterson, J. Goldberg Northwestern Univ. Med. School, Dept. Physiology and Rehab Med., Chicago IL 60611

By imagining a target rotating with them, human subjects (Ss) can suppress the vestibulo-ocular reflex (VOR) elicited by sinusoidal whole body rotation in the dark. This suppression (SUP) is thought to be mediated either by the smooth pursuit system (P) or by an independent predictive process (Robinson in Functional Basis of Ocular Motility Disorders. Lennerstrand et al 1982). In either case SUP should be less effective for pseudorandom rotations than for the predictable sinusoidal rotations used in the past.

As illustrated by the data below, we have examined the response of the SUP and P systems to an unpredictable stimulus (SSN) consisting of a sum of 5 sinusoids (0.2-1.9Hz) and found that while the P response gain is less than that obtained with (0.1-2.5Hz) sinusoids (SIN), the gain of SUP is unchanged. Eye movements were recorded by DC electrooculography, desaccaded and differentiated to obtain the velocity of smooth phase eye movements (VSE). The VSE obtained when Ss tracked a moving 1cm light spot at 1.5m distance was divided by spot velocity (85° /s) to obtain P gain. For SIN stimuli, this gain was 0.9-1.0 for frequencies up to 0.5Hz and then fell rapidly; for SSN, gain was low except at 0.2Hz. VSEs measured during 90°/s rotation in the dark with subjects instructed to relax (rVSE) or suppress (sVSE) were used to compute gain and phase of the SUP transfer function by performing the following operation vectorially:
SUP = (rVSE-sVSE)/rVSE. SUP gain was ~ 0.6 for SIN and SSN up to 1Hz, after which it declined for SIN.

These differences in gain behavior for SIN and SSN stimuli, which were observed in 4 normal Ss, indicate that pathways mediating SUP and P are not identical. The difference in phase behavior of the two processes shown at the lower right also supports this conclusion. The tendency of both SUP and P gains to fall off above 1Hz, especially for SIN stimuli which had higher accelerations, suggests however, that the two may share some common elements.

Supported by Coleman, Hearst, J.M., Joyce and Searle Foundations.



250.11 MECHANISMS UNDERLYING THE INTERACTION OF THE VESTIBULO-OCULAR REFLEX (VOR) AND SMOOTH PURSUIT (SP) IN MONKEY. S.G. Lisberger, Dept. Physiol., Div. Neurobiol., UCSF, San Francisco, CA 94143

Two models can account for the eye movements generated when eye and head movements are used to track a smoothly moving target: 1) a "linear addition" model, in which eye movements are produced by adding two velocity commands, one from the VOR and one from SP; 2) a "parametric control" model, in which tracking conditions momentarily alter transmission through VOR pathways.

We have discriminated between these models by a) placing the eye-head tracking system in modes that should bring out any parametric control, and b) using rapid steps of head or target velocity to make direct measures of the gain and latency of SP and the VOR in these different modes. Experiments consisted of a series of trials, each of which began with the monkey tracking a small target during passive head motion at 15°/s. Initial conditions were established by the mode of coordination between target and head. If the target moved with the head ("times zero" or x0-tracking), it was tracked by keeping the eyes fixed in the orbit; this could be done by momentarily reducing transmission through VOR pathways (parametric control) or by allowing the VOR to remain active and counterbalancing it with a command for SP (linear addition). If the target moved opposite to the head (x2-tracking), it was tracked by rotating the eyes at twice head velocity; this could be done by doubling transmission in VOR pathways, or by adding a command for SP to a normal VOR.

To measure the performance of the VOR, we have imposed step changes in head velocity at unexpected times, and averaged the eye velocity responses to at least 20 identical stimuli. The latency of the VOR was 14-15 ms and did not depend on initial tracking conditions. Superimposing the averaged responses from different initial conditions showed that the first 40 ms of the VOR was the same during both x0- and x2-tracking. However, differences became apparent 40-60 ms after the step of head velocity: the eye velocity of the VOR was up to 30% larger during x2- than during x0-tracking. These differences persisted if visual errors were eliminated by establishing open-loop tracking conditions for 200 ms before and after the change in head velocity. To measure the performance of SP, we have imposed step changes in target velocity. The latency of SP was 100-120 ms and did not depend on initial conditions; however, the open-loop gain of SP was up to 25% greater during x2- than during x0-tracking.

We conclude that the VOR is always active at a gain close to that measured during head motion in the dark and that combined eye-head tracking is effected largely by the addition of independent velocity commands from the VOR and SP. However, the gains of both commands are under some parametric control. (Supported by EY03878 (NIH) and by the McKnight and Sloan Foundations)

250.12 ATTENUATION OF THE OTOLITH-OCULAR TORSION REFLEX BY CHANGING ORIENTATION WITH RESPECT TO GRAVITY.
A. P. Arrott* and L. R. Young.
Man-Vehicle Laboratory, M.I.T., Cambridge, MA 02139.

Lateral tilt of the head in a gravitational field produces an ocular counterrolling response. Compensatory eye roll is in a direction to stabilize the visual field on the retina.

The indistinguishability of gravitational and inertial forces results in an ocular torsion response to lateral acceleration in a subject with his head upright. In this case, the gravitoinertial force (GIF) vector rather than the pure gravitational force vector is rotated with respect to the head. Furthermore, the centrifuge experiments of Woellner and Graybiel [J. Appl. Physiol., 14:833, 1959] suggest and the parabolic flight experiments of Arrott and Young [Soc. Neurosci. Abstr., 7:484, 1981] verify that a change in the lateral component of the GIF vector, rather than a rotation of the GIF vector, is a sufficient stimulus for ocular torsion.

This evidence supports the notion that retinal stabilization by ocular counterrolling is achieved by a simple reflex arc in which lateral GIF acting on the otoliths elicits ocular torsion. This is in contrast to a central integration view that sensory information from the otoliths for all of three dimensional space is integrated into an estimate of head orientation before such information is used to determine motor outflow. An experiment was devised to place the two notions in sharp contrast.

Using a linear acceleration cart, human subjects are accelerated laterally while lying supine. The lateral component of GIF sufficient for eliciting ocular torsion is present. However, the net rotation of the GIF vector is around the longitudinal axis of the subject and ocular torsion is an inappropriate compensatory response. In contrast, for lateral acceleration of the upright subject, even though the lateral component of GIF is identical, the rotation of the GIF is in the frontal plane, for which ocular torsion is appropriate. Therefore, in comparing the three cases of lateral acceleration: in upright, zero gravity, and supine conditions; we might expect ever decreasing support for ocular torsion from a central integration point of view. Measurements of the amplitude of ocular torsion in these conditions indicates that whereas the upright response is comparable to the analogous lateral head tilt, the supine response is reduced by a factor of three.

The results imply that a dominant simple reflex arc from lateral GIF to ocular torsion is capable of being attenuated but not eliminated by otolith information from other axes which deem the ocular torsion inappropriate for retinal stabilization.
(supported by NASA grants NAS9-15343 and NSG-2032).

250.13 DO THE EYES COUNTERROLL DURING BARBECUE ROTATION? Charles H. Markham and Shirley G. Diamond. Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

It has been known for 200 years that the eyes counterroll in response to roll about the naso-occipital axis. This reflex is called ocular counterrolling (OCR), and is now thought to be governed by the otolith organs in the inner ear, specifically the utricles responding in a reciprocal manner to the shear force exerted by gravity as they are tilted from the horizontal plane. When a subject is tilted to 90° in our laboratory's dynamic roll protocol, his eyes typically will counterroll about 6°. However, his head would be in the same final location if he had been turned on his side from a supine position. Does OCR occur during this maneuver? Might barbecue (BBQ) rotation, i.e. head and body longitudinal axis turning earth horizontal, be a means of inferring saccular function?

To examine these questions, 3 normal subjects and 4 patients with partial or complete unilateral vestibular nerve sections were tested in both roll and BBQ protocols. Roll consisted of tilting persons about their naso-occipital axes to 90° right ear down at a constant velocity of 3°/sec, held there for 30 sec, rolled to the opposite side to 90° left ear down, held 30 sec, and brought back through the upright position (Trial I). Without stopping the procedure was repeated (Trial II). BBQ rotation consisted of placing subjects in the supine position and turning them from nose up to 90° right ear down at a constant velocity of 3°/sec, holding them for 30 sec, returning them to nose up, held for 30 sec (Trial I), and repeating the procedure (Trial II). This was then performed for left ear down position. Acceleration and deceleration were calculated at 0.21°/sec², considered to be below the threshold of the semicircular canals. Photographs were taken of both eyes at each 10° of rotation. Measurements of OCR were made with a two-projector apparatus using a superimposition technique which is accurate to 0.25°.

Results showed that OCR does occur in BBQ rotation, and that the OCR in a subject's BBQ rotation closely resembles his OCR in roll, although usually somewhat attenuated in amplitude. Patients with unilateral vestibular nerve sections showed the same abnormalities in both modes, namely lack of consistency between trials, and disturbances in smoothness and symmetry. Tilted to the side of the lesion, OCR was normal; tilted to the intact side, OCR was disrupted. Patients who had sections of only the superior branch of the vestibular nerve showed the same OCR abnormalities as did patients with complete nerve sections. This would suggest that the utricle is responsible for OCR in BBQ rotation as well as in roll, since the preserved saccular function in patients with intact inferior branch was not sufficient for normal OCR.

250.14 ADAPTIVE PLASTICITY IN SHORT-LATENCY OCULAR FOLLOWING RESPONSES OF MONKEY. K. Kawano* and F. A. Miles. Lab. Sensorimotor Res., National Eye Institute, Bethesda, Md 20205.

The initial ocular following response to a sudden drifting movement of the visual scene is generated open-loop, and its magnitude is a function of the gain of the visual tracking system. If the gain were high, the eyes would tend to overshoot the moving scene, while a low gain would result in undershoot. To ascertain whether the gain is subject to adaptive regulation, we have examined the effects of repeated exposures to brief velocity-step movements of the scene that first initiate ocular following and then transiently induce retinal events resembling those expected during undershoot or overshoot.

Monkeys faced a screen subtending slightly less than 90° and their eye movements were recorded using the electromagnetic search coil technique. A random dot pattern was back-projected onto the screen via an x-y mirror galvanometer system under computer control. Initial tracking responses to movements of the scene were assessed using 100ms test ramps, randomizing time of onset, direction, and speed (10-100°/s). Without training or reinforcement, alert animals responded with unexpectedly short latencies of almost machine-like consistency. Latencies for 2 animals: means, 52ms & 53ms; ranges, 46-62ms & 45-63ms; average SD's for responses to given ramps, 4.7ms & 4.1ms.

During the velocity-step sequences used for adaptation, the scene was first drifted for 150ms in one of 4 directions at one of 7 speeds (10 to 100°/s); drift speed was then either stepped up to 100°/s (causing the eye to "undershoot" the moving scene) or, in other experiments, stepped down to 0°/s (causing "overshoot"), for a further 150ms. Each sequence was terminated by blanking the screen for 0.5s. (Initial velocities and inter-trial intervals varied randomly.) Over a 3-day period, clear adaptive changes were seen with both paradigms in both animals: After step-up experience, peak eye speeds elicited by 100ms test ramps increased on average by 74% & 92% (ranges: 41-91% & 4-224%), and after step-down they decreased on average by 49% & 61% (ranges: 42-63% & 56-65%). In the low-gain state, eye velocity response profiles began to deviate from controls at latencies of 60-70ms, while high-gain responses did not deviate significantly until 90ms or more after stimulus onset: The very earliest responses never altered and for at least the first 40ms responses were near maximal in the normal animal. Adaptation was also directionally selective: Responses in any one of the primary directions up, down, left or right, could be altered independently. Furthermore, if the velocity steps involved 90° changes in the direction of movement (speed constant), tracking responses to 100ms test ramps acquired corresponding orthogonal components. We conclude that ocular following response vectors are subject to extensive, visually-mediated adaptive regulation.

250.15 POST-SUPPRESSION VESTIBULO-OCULAR REFLEX (VOR) IN MAN: NON-PURSUIT VOR CANCELLATION? Bernard N. Segal. Aviation Medical Research Unit, Dept. Physiol., McGill Univ., Montreal, Canada H3G 1Y6.

When the head is turned while following an object that moves with the head, the normally compensatory action of the VOR is inappropriate and requires suppression. To clarify whether the pursuit system participates in such suppression, the present study examined VOR suppression during whole-body sinusoidal and triangular (0.01-0.17 Hz, 60°/s) oscillation about the vertical axis while subjects fixated on a stationary (re: head), intermittently-illuminated (light-on: 9 s; light-off: 2 s) point. The relative contribution of pursuit to VOR suppression was then estimated by comparing slow-phase eye velocity elicited when the fixation light was on (pursuit present) with eye velocity seen just after the light was extinguished (pursuit absent). To prevent the appearance in darkness of pursuit-afternystagmus (Vis. Res. 19: 1057, 1979), which normally could result from previous charging of velocity storage processes by efference copy, darkness intervals were chosen to be too brief for efference copy to activate sluggishly responding velocity storage processes. Furthermore, during target fixation, efference copy and full-field retinal slip, associated with residual eye movements, should all be too small for significant activation of velocity storage. METHODS: Because the frequency of darkness intervals was always a non-integer multiple of the frequency of body oscillation, darkness intervals appeared at different phases of successive cycles of body oscillation. Stimulus-locked averages of slow-phase eye velocity at the end-portions of all darkness intervals were suitably obtained, thereby providing an estimate of the 'post-suppression VOR'. To compare the post-suppression VOR with the normal VOR in maintained darkness, the fixation light was extinguished for about 1 min immediately following each 2-min test of intermittent VOR suppression. Six subjects were tested. RESULTS: When compared to the normal VOR in maintained darkness, the post-suppression VOR (1) was 25-45% smaller at all oscillation frequencies, and (2) exhibited larger phase shifts during lower frequency sinusoidal oscillation. When the fixation light was extinguished following intermittent suppression, the gain of the VOR required 6-30 s to build up to normal values (even in naive subjects). These latter values did not show any progressive adaptive attenuation over the course of experiments. These results suggest (1) that VOR suppression is achieved by BOTH pursuit and non-pursuit processes, and (2) that the non-pursuit processes, presently of unknown origin, are responsible for about 55-75% of this suppression. Finally, it is hypothesized that these non-pursuit processes might employ signals derived from the semicircular canals.
(Supported by McGill Fac. Med. and Canadian Medical Research Council).

250.PO UNILATERAL CEREBRAL CORTICAL ABLATIONS IMPAIR VESTIBULO-OCULAR REFLEX (VOR) GAIN AND PLASTICITY IN THE CAT. J. L. Demer*, R. J. Tusa and S. J. Herdman. Wilmer Eye Inst., Johns Hopkins Hosp., and Univ. of Maryland, Baltimore, MD 21205.

Gain (slow-phase eye velocity/head velocity) of the VOR may be plastically modified by prolonged abnormal visual-vestibular experience, such as chronic wearing of magnifying spectacles or artificially induced motion of the visual field during head rotation. To study this plasticity, we measured VOR gain in 6 adult cats using the magnetic search coil in response to 30 and 60 deg/s head velocity steps and during sinusoidal head rotation in the dark at 0.05 or 0.25 Hz (± 30 deg/s). Animals were then exposed to 2-3 hrs of visual experience with a full-field optokinetic drum moving sinusoidally at the same velocity as the head but 180 deg out of phase. Following this, VOR gain was increased in all cats, typically by 0.15 to 0.40 for both left and right eye velocities. The optokinetic drum was then rotated in-phase with head velocity for an additional 2-3 hrs. Following this, VOR gain was decreased for both directions of eye velocity in all cats, by 0.15 to 0.80. These plastic gain changes are similar to those induced by magnifying or reversing spectacles.

Unilateral cortical lesions were made by subpial aspiration in anesthetized animals. Two cats underwent complete removal of visual cortex in the left hemisphere (VC); two underwent partial removal of the medial bank of the lateral suprasylvian sulcus (LS); one underwent removal of areas 17 & 18 on the left (17/18); and one underwent removal of areas 17 & 18 on the left plus corpus callosum section (17/18 + CC). VOR gain plasticity studies were repeated 2 or 3 times in each animal at 7 to 68 days post-operatively. Lesions were verified histologically.

VOR gain for eye velocities to the right was not significantly changed post-lesion, but was persistently decreased by 10-50% for eye velocities to the left (towards the lesion). The degree of reduction depended on the type (step or sine) and frequency of stimulus. Similarly, VOR gain plasticity for eye velocities to the right was unchanged post-lesion, but was persistently impaired for eye velocities to the left in the cats with VC and LS lesions. VOR gain and plasticity were unchanged in cats with 17/18 and 17/18 + CC lesions. Peak eye velocity in response to an optokinetic drum accelerating at 0.27 deg/sec² was persistently decreased to the left by about 50% in cats with VC, and 17/18 +CC lesions, and was transiently decreased by the same amount in cats with LS lesions.

These preliminary results indicate that cerebral cortical lesions can impair VOR gain and plasticity in a directionally-selective manner independent of effects on the optokinetic system.

251.1 HEAVY CELL LOSS IN DENTATE NUCLEUS OF THE MONKEY FOLLOWING NEONATAL ABLATION OF THE CEREBELLAR CORTEX. E. Meisami (SPON: G. Westheimer). Dept. of Physiol.-Anat., Univ. of Calif., Berkeley, California 94720

The dentate nucleus receives its most prominent input from the Purkinje cells of the neocerebellar cortex. Quantitative morphometric studies and cell counts were conducted in the left and right dentate nuclei of four adult Macaque monkeys that had been subjected to unilateral ablation of the cerebellar cortex during the early postnatal period. Examination of serial, celloidin embedded, Nissl stained, 25 u thick sections confirmed massive absence of the cortex of neocerebellar hemisphere on the operated side with no damage to the contralateral (control) side. Comparison of the dentate nucleus deprived of the cortical input with the contralateral dentate (unoperated control) revealed highly significant reductions not only in the total volume (40-80%), total number of large (20-40%) and small (40-55%) neurons, but also in the size of the remaining cells (5-20%). The loss in cell number was less marked when the lesion had spared the nucleus completely. The higher reductions in volume relative to cell number resulted in increased cell densities in the deafferented dentate. The remaining cells of the latter appeared normal, however, in other respects. These results indicate that in the postnatally developing monkey, the survival of a significant number of dentate neurons and the proper growth of others are critically dependent on some influence from the Purkinje cells of the neocerebellar cortex.

251.2 Climbing Fiber Projections from Physiologically Identified Areas of the Cat Dorsal Accessory Olive A. R. Gibson, J. C. Houk, and F. R. Robinson. Physiology Dept., Northwestern Univ. Med. Sch., Chicago, Ill. 60611.

The rostral dorsal accessory nucleus of the inferior olive (rDAO) contains a detailed map of the entire contralateral body surface (Gellman et al., J. Comp. Neurol., 215:228-243, 1983). Fibers from rDAO project to the anterior interpositus nucleus (NIA) of the cerebellum. Forelimb and hindlimb regions of NIA have been identified by tracing the interpositus connections to the magnocellular division of the red nucleus (RNm). We examined the projection from rDAO to the NIA to determine if the forelimb and hindlimb regions of rDAO project to the corresponding forelimb and hindlimb regions of NIA. We also studied the projection from rDAO to the cerebellar cortex to identify the regions that receive forelimb and hindlimb information from rDAO.

Small amounts (≈0.008 µl) of 1% wheatgerm agglutinin conjugated horseradish peroxidase (WGA-HRP) were pressure injected into physiologically identified forelimb and hindlimb areas of rDAO. We traced the anterogradely labeled fibers and terminals into the cerebellar nuclei and cortex. Injections into the forelimb region of rDAO labeled terminals only in the region of NIA that projects to the forelimb division of RNm. Injections into the hindlimb region of rDAO labeled terminals only in the area of NIA that projects to the hindlimb division of RNm.

Climbing fibers from the forelimb and hindlimb areas of rDAO terminate in largely nonoverlapping regions of the cerebellar cortex. Consistent with earlier studies, fibers from the forelimb area of rDAO terminate in parasaggital stripes in the posterior regions of the anterior lobe and in the rostral folia of the paramedian lobule. Fibers from hindlimb rDAO terminate in parasagittal stripes in more anterior regions of the anterior lobe and in the caudal folia of the paramedian lobule. Descriptions of cortico-nuclear projections suggest that the cortical areas receiving forelimb or hindlimb information from rDAO project to the corresponding fore- or hindlimb region of NIA.

The alignment of sensory input from rDAO with the motor output somatotopy in NIA suggests that the sensory information in rDAO influences movements of the body part from which the sensory information arises.

251.3 BASAL INTERSTITIAL NUCLEUS OF THE CEREBELLUM: A DEEP CEREBELLAR NUCLEUS RELATED TO THE FLOCCULUS. T.P. Langer* (SPON: C.R.S. Kaneko) Dept. of Physiology and Biophysics and Regional Primate Center, University of Washington, Seattle, WA 98195

In the course of studying the afferents and efferents of the flocculus in the rhesus monkey, it became apparent that a previously undescribed deep cerebellar nucleus exists that is reciprocally interconnected with the flocculus. In the monkey the flocculus is not connected with any of the major deep cerebellar nuclei, but there is an interstitial population of neurons in the white matter ventral to the deep cerebellar nuclei and extending into the peduncle of the flocculus that have reciprocal connections with the flocculus. This collection of neurons will be called the basal interstitial nucleus of the cerebellum.

Several lines of evidence indicate that the basal interstitial nucleus is a separate, distinct and complete nucleus. (1) Injection of HRP into the flocculus always labels a group of neurons immediately ventral to the deep cerebellar nuclei and extending posteromedially into the lateral margin of the nodulus and rostrolaterally around the caudal aspect of the y-group to infiltrate the peduncle of the flocculus. (2) In Nissl-stained material there is a readily seen nucleus, clearly distinct from the overlying cerebellar nuclei, with precisely the same distribution. The neurons in the basal interstitial nucleus of the cerebellum have a characteristic morphology. They are intermediate size chromatophilic multipolar neurons with a fusiform shape and rapidly tapering proximal dendrites. The cell nucleus is generally eccentrically placed in the cell body, against the cell membrane or in one pole of the cell. The Nissl substance is usually finely granular in the center of the cell body and forms dense clumps adjacent to the plasma membrane. (3) Anterograde label from injections of HRP or tritiated amino acids in the flocculus extends over the entire basal interstitial nucleus. In one brain with an HRP injection involving a part of the basal interstitial nucleus there was a patchy clustered distribution of labeled neurons extending throughout the flocculus and into the adjacent lateral parts of the simple lobules. The clusters tended to be confined to the medial half of many of the folia.

These observations suggest that a separate previously undescribed deep cerebellar nucleus exists in the monkey that interacts reciprocally with the flocculus and perhaps with parts of the simple lobules.

(Supported by EY-03212)

251.4 Distribution of Terminals in Cerebellar Cortex and Their Relative Absence in the Deep Cerebellar Nuclei from Three Major Sources of Mossy Fibers in Cat. R. Chen*, A.R. Gibson, J.C. Houk, and F.R. Robinson. Physiology Dept., Northwestern Univ. Med. Sch., Chicago, Ill. 60611.

Injections of WGA-HRP were made in the external cuneate nucleus, the lateral reticular nucleus, and the pontine nuclei of the cat. Sections were then reacted with a modified TMB procedure which allowed clear visualization of anterogradely labeled fibers and terminals. Cortical label was restricted to the granular layer and demonstrated mossy fiber glomeruli. The cortical distribution of labeling, in general, agreed well with previous investigations. Injection into the external cuneate nucleus labeled terminals in the medial and intermediate portions of both anterior and posterior lobe cortex. Injection into the lateral reticular nucleus labeled intermediate portions of anterior and posterior lobe cortex. The pontine nucleus injection labeled lateral and intermediate portions of the posterior lobe. The paramedian lobule is the only region of the cerebellum to receive input from all three mossy fiber sources.

The mossy fiber input to the cerebellar cortex is often considered to be diffuse but, in these experiments, it shows evidence of a high degree of specificity. The lateral reticular and external cuneate injections produced sharply delimited longitudinal borders between labeled and unlabeled regions. Following these injections the medial and intermediate portions of the anterior lobe contained several distinct longitudinal columns of labeled glomeruli.

It is generally assumed that cells which provide a mossy fiber input to cerebellar cortex also send an axon collateral to cells within the deep nuclei. Since the pontine nuclei, external cuneate, and lateral reticular injections each label a massive number of fibers leading to cerebellar cortex, it might be expected that a large projection to the deep nuclei would also be present. No such projection was found. Labeled fibers from the pontine injection passed laterally to the deep nuclei with no visible branching to them (Dietrichs et al., Br. Res., 259:127-131, 1983, report similar findings). Most of the labeled fibers from the external cuneate and lateral reticular injections coursed through the white matter surrounding the deep nuclei. Only in the case of the lateral reticular injection could terminals clearly be seen. They were lightly distributed within the interposed nuclei and clustered around the cell somas. The results indicate that the cerebellar cortex receives a heavy mossy fiber input that, for the most part, is not shared with the deep nuclei.

251.5 ANATOMIC CHARACTERISTICS OF PONTINE-PROJECTING NEURONS WITHIN THE DORSAL COLUMN NUCLEI OF THE CAT. J.G. May, III* and K.J. Berkley. Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306.

Recent anatomic studies have revealed efferent projections from the dorsal column nuclei (DCN) to the pontine grey in the rat (Kosinski et al., '82) and cat (Björkeland & Boivie, this vol.). The purpose of the present study was to characterize the cells of origin of this DCN-pontine projection anatomically.

Small injections of wheatgerm agglutinin:horseradish peroxidase conjugate or tritiated N-acetyl wheatgerm agglutinin were placed in the DCN-recipient regions of the pontine n. In all four cases, the majority of labeled neurons formed a band around the medial and ventral edges of the DCN complex. This band began by extending down the most medial aspect of the gracile n., bending laterally as it reached the basal region, and then continuing laterally through the basal portions of the cuneate n. A small number of labeled neurons were seen scattered within the dorsal portions and the rostral poles of DCN, as well as a few in the deep regions of the spinal trigeminal nucleus. Most of the labeled neurons were small and triangular, fusiform or multipolar in shape. Within the dorsal portions of DCN, only very few of the large, round "cluster" cells that are labeled after thalamic injections (Berkley, '75) were labeled by the pontine injections. Most labeled neurons in this region were large stellate or small fusiform cells.

The anatomic characteristics of DCN neurons projecting to the pons are different from those of neurons projecting to the thalamus, but similar to those of neurons projecting to the cerebellum, spinal cord, pretectum and tectum (Cheek et al., '75; Rinvik & Walberg, '75; Burton & Loewy, '77; Berkley et al., '80; Bull & Berkley, '82; Budell & Berkley, this vol.). Thus it appears that the pontine-projecting neurons may represent a distinct subset of a population of neurons that are located around the edges of and between the gracile and cuneate n. and that do not project to the thalamus.

A number of older as well as recent anatomic and electrophysiological studies indicate that the ventral portions of DCN receive considerable input from muscles and other deep structures. The present results thus suggest that information from these structures may be relayed to the cerebellum from a distinct population of DCN neurons by way of the pons. These projections along with the previously-reported projections from a similar group of DCN neurons directly to the cerebellum, pretectum, tectum and spinal cord (and perhaps other regions such as the red n., inferior olive and zona incerta) may form part of a complex efferent system from DCN that is functionally related to the fine control of proximal body, or head and neck movement.

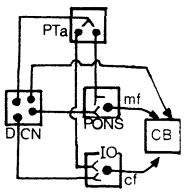
Supported in part by PHS grant RO1-NS-11892 from NINCDS.

251.6 INTRICATE RELATIONS BETWEEN THE DORSAL COLUMN NUCLEI, PRETECTUM, INFERIOR OLIVE, PONS AND CEREBELLUM IN THE CAT. M. S. Bull, J. G. May, III*, R. J. Budell* and K. J. Berkley. Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306.

In addition to their dense projections to the thalamus, the dorsal column nuclei (DCN) have a less well known set of projections to the cerebellum. These projections are not only direct, but also indirect, involving a climbing fiber route through specific portions of the inferior olive and a mossy fiber route through specific portions of the pons (Björkeland & Boivie this vol.; May & Berkley, this vol.).

Recent work has shown that neurons within another of DCN's targets, the anterior pretectal nucleus, project their axons to the DCN-recipient portions of the inferior olive (Mitchell et al., '81). The purpose of the present study was to determine whether the DCN-recipient portions of the pons likewise receive an input from the DCN-recipient portions of the pretectum.

After injecting wheatgerm agglutinin conjugated to horseradish peroxidase in the vicinity of the DCN-recipient portions of the pons and processing the tissue two days later with tetramethylbenzidine, retrogradely labeled neurons (but no anterogradely labeled terminals) were observed in the anterior pretectal n. (PTa). When injections of ^{3}H-leucine were placed in PTa, terminal labeling was seen in a region adjacent to and overlapping the DCN-recipient portions of the medial pontine n. In subsequent experiments, ^{3}H-leucine injections were placed into the PTa of cats that had also received DCN lesions. The autoradiographically-visualized pretectal terminations in the pons encompassed the region containing degenerating DCN terminations. These results indicated that, like DCN-recipient portions of inferior olive, DCN-recipient portions of the pons receive DCN-modulated input by way of the pretectum.



In conjunction with the results of studies in this and other laboratories demonstrating that the population of DCN neurons projecting to the pretectum, inferior olive, pons and cerebellum are distinctly different anatomically from those projecting to the thalamus (e.g. May & Berkley, this vol.), the present results suggest that the pretectally-influenced and DCN-modulated climbing fiber and mossy fiber routes to the cerebellum may be related to postural mechanisms or to the coordination of proximal body or head and neck movement.

Supported by PHS grant RO1-NS-11892 from NINCDS.

251.7 A DOUBLE LABEL RETROGRADE TRACING STUDY OF THE OLIVOCEREBELLAR PROJECTION TO THE PYRAMIS AND UVULA IN THE RAT, L. M. Eisenman and G. P. Goracci. Daniel Baugh Institute, Jefferson Medical College, Philadelphia, PA 19107

Recent electrophysiological (Faber & Murphy, 1969 and others) and anatomical (Brodal, et al., 1980) studies of the organization of the olivocerebellar projection in the cat suggests that climbing fiber collaterals innervate different parts of the same parasagittal zone and/or different parasagittal zones (Ekerot & Larson, 1982). This finding is not surprising when one considers the numerical mismatch between inferior olivary cells and Purkinje cells in this and other species (Escobar et al., 1968). In an attempt to determine whether climbing fiber collaterals innervate neighboring folia in the posterior lobe of the cerebellum in a different species, the rat, a double labeling experiment was undertaken. Based on previous HRP studies of the olivocerebellar projection to the uvula and pyramis in the rat, a zone within these folia which receives an input from subnucleus β of the caudal medial accessory olive (MAO) was chosen for further study. Separate microinjections of the fluorescent tracers nuclear yellow (NY) and fast blue (FB) were made in the medial zone of the uvula and the intermediate zone of the pyramis. As mentioned HRP studies had demonstrated that these two regions receive an olivary input from the caudomedial MAO and primarily subnucleus β. After a 24-hour survival time the rats were sacrificed and 30μ frozen sections were prepared for examination with a Zeiss epifluorescent microscope using a 48-77-02 filter system that provides an excitation wavelength of 365 mm.

In these experiments it was determined that most cells within nucleus β were labeled with either NY or FB. A third, smaller population of olivary cells in nucleus β contained both NY and FB. We could not detect any segregation of these three populations of cells within the caudomedial MAO. We conclude from these data that although these two cortical sites are not aligned with one another, they are part of the same parasagittal zone in that they get input from the same olivary region. Most of the olivary cells in the caudomedial MAO project to only one or the other of these cortical sites whereas a smaller population of olivary cells in this same area have climbing fiber collaterals which innervate both cortical regions. These three populations are not spatially segregated.

251.8 HYPOTHALAMO-CEREBELLAR PROJECTIONS IN THE SQUIRREL MONKEY (SAIMIRI SCIUREUS): AN HRP STUDY. D.E. Haines and E. Dietrichs*. Dept. of Anatomy, West Virginia Univ., Morgantown, W.Va. 26506, and Anatomical Institute, Univ. of Oslo, Oslo, Norway.

Although it has been suggested that the cerebellum may directly influence, or be influenced by, autonomic centers, the anatomy of these potential connections remains enigmatic. Recently Dietrichs (Science, 1983, in press) has described a direct connection from hypothalamus to cerebellar cortex in cat. The present study provides evidence of a hypothalamo-cerebellar projection in squirrel monkey.

Injections of horseradish peroxidase (Sigma, VI HRP in 35% solution; WGA-HRP in 2.5% solution) were made into the cortex of the paraflocculus and into dorsal paramedian, ansiform and dorsal culminate lobules using iontophoretic (HRP) and/or pressure (WGA-HRP) methods. Following survival times of 22-50 hours the animals were killed by transcardiac perfusion with an appropriate fixative followed by a wash solution. Hypothalamus, brainstem and cerebellum were cut in frozen section at 50μm and processed using tetramethylbenzidine as the chromogen. In no case did cortical injections involve the cerebellar nuclei.

In all experiments (save one dorsal culminate case) retrogradely labeled cells are present in caudal hypothalamus; the distribution is bilateral with a slight ipsilateral preponderance. Following the terminology of Emmers and Akert (1963) labeled cells are found primarily in posterior (APH) and lateral (ALH) hypothalamic areas, the supramammillary nucleus (SmH) and in the immediate area of the mammillothalamic tract. These latter cells may be displaced somata from SmH or ALH. Isolated, yet well labeled, cells are occasionally seen in the medial mammillary nucleus following injections of paraflocculus. No labeled cells are present in the adjacent subthalamic nucleus or substantia nigra; the occasionally labeled cells seen in the medial edge of the internal capsule are presumably displaced from ALH. In an effort to assess the possible reciprocity of this pathway pressure and iontophoretic injections were made into caudal hypothalamus. Following iontophoretic injections, largely restricted to ALH and APH, sparse numbers of HRP labeled cells are seen bilaterally in ventral areas of lateral and posterior interposed cerebellar nuclei. These results indicate a direct bilateral connection between caudal hypothalamus and the cerebellar cortex in this primate. Preliminary data from hypothalamic injections further suggests that some of the cerebellar nuclei project directly, and bilaterally, to caudal hypothalamus.

(Supported by NIH grant NS11327 from NINCDS (DEH)).

251.9 CEREBELLAR AFFERENTS IN THE MUTANT MOUSE WEAVER AS STUDIED BY RETROGRADE HRP INJECTIONS. D.E.Smith. Department of Anatomy, L.S.U. Medical School, New Orleans, LA 70112.

The disappearance of the granule cells in the neurological mutant mouse weaver leads to a cortical disarray in the cerebellum (Rakic, P., and R.L. Sidman, Proc. Nat. Acad. Sci. 70: 240, 1973) and retrograde changes within spinal cord nuclei that send afferents to the cerebellum (Smith, D.E., et al., Neuroscience Lett. 28: 175, 1982). In light of the retrograde changes seen in the spinal cord, studies were initiated to determine what, if any, alterations were present in other neuronal systems that project to the cerebellum.

In order to first establish whether or not there are any aberrant projections as a result of the loss of cerebellar granule cells, studies were begun to ascertain the location of neurons that project into the granule layer of the cerebellum in the mutant mouse weaver and compare these results with comparable injections into homozygous and heterozygous controls.

Breeding pairs of weaver mutants (B6CBA-A^{WJ}/A-wv) were obtained from The Jackson Laboratory and maintained in our vivarium. Offspring, ranging in age from 4 weeks to 4 months, received cerebellar injections of 0.2 µl of a 20% aqueous HRP solution at a depth of 600 microns. Following a 24 hour survival period, the animals were anesthetized and perfused with 1% paraformaldehyde - 1.25% glutaraldehyde solution in a 0.1 M phosphate buffer. The brain stem and cerebellum were subsequently removed, sectioned midsaggitally and processed for HRP enhancement according to the de Olmos technique (de Olmos, J.S., Exp. Brain Res. 29: 541, 1977).

Injections into lobules V and VI of the vermal region resulted in labeling of neurons within the following nuclei: lateral cuneate, hypoglossal, accessory olive, principal sensory, pontine, dorsal and ventral cochlear, and nucleus of the trapezoid body. Preliminary observations suggest that the major difference between the origin of cerebellar afferents in the mutants and the homozygous and heterozygous controls is one of the number of neurons that project to the cerebellum rather than the origin of the neuronal projections. These findings suggest the presence of an active neuron-target relationship in the postnatal development of those brain stem nuclei which have extensive cerebellar projections.

This work is supported by N.I.H. grant NS 13155.

251.10 ORIGIN OF THE CEREBELLAR TRANSCOMMISSURAL SYSTEM. A.Rosina* and L.Provini*(SPON: European Neuroscience Association), C.N.R. , Ist.Fisiol.Centri Nervosi,Via Mario Bianco 9,20131 Milano,Italy.

It was recently shown that neurons located in corresponding regions of the right and left pontine nuclei (PN) project by way of branched axons to common bilateral target areas of the cerebellum. Cerebellar midsagittal transection experiments,moreover, indicated that this axonal branching takes place within the cerebellum (Rosina et al.,Brain Res.195 ,461-466,1979).Thus this fiber system appears to be a transcommissural link between the two cerebellar hemispheres.

To further analyze the relative incidence and the origin of these fibers, we injected two spectrally different fluorescent tracers into the opposite cerebellar hemispheres of cats, one before and one after cerebellar midsagittal transection. In this way the first tracer retrogradely labels all the PN neurons projecting to one cerebellar hemisphere, while the second tracer selectively labels those PN neurons whose axons project directly, and not by crossing within the cerebellum,to the other hemisphere

The results of 5 such experiments showed that the neurons retrogradely labeled by the first tracer were located bilaterally within the caudal two-thirds of the PN . On the contrary, the neurons retrogradely labeled by the second tracer (injected after the cerebellar transection) were mainly localized contralaterally to the injected site, with the exception of labeled neurons in the dorsolateral nucleus.Within the areas in which the two labeled populations overlapped,double-labeled neurons were seen. The bilaterally projecting neurons were found throughout the caudal two-thirds of the PN, located in the peripeduncular , ventral , lateral and dorsolateral subdivisions, in which they could reach the incidence of 50%. Moreover the double-labeled neurons on the side ipsilateral to the first tracer injection (re-crossing neurons) greatly outnumbered the double-labeled neurons on the opposite side (crossing neurons) by an average of 9 to 1.

On the whole our data indicate that the bilaterally projecting transcommissural neurons represent 5-10% of the entire population of PN neurons projecting to the lateral hemisphere. The system is mainly composed of PN neurons whose axons re-cross the midline within the cerebellum after crossing within the brain-stem.

251.11 DEVELOPMENT OF CEREBELLOBULBAR CONNECTIONS IN THE POUCH YOUNG OPOSSUM. James C. Hazlett and Stuart D. Bagley* Department of Anatomy, Wayne State University, Detroit, MI 48201.

The present study is the third in a series of reports which emphasize significant details in the development of the long ascending somatosensory and sensorimotor pathways. Early stages in the development of cerebellar projections to the vestibular nuclei, inferior olivary complex and basilar pons were studied in litters of pouch young opossums 9, 12, 16, 20 and 26 days of age. Hemicerebellectomies performed in the 12 through 26 day old groups were followed by 18-24 hour survivals. The animals were sacrificed under hypothermic anesthesia with formol-saline and the brains were processed for degenerating developing axons with the Fink-Schneider '69 technique. In the youngest group (9 days old) tissue from cerebellar HRP injections was examined for the presence of cerebellofugal fibers. In these preparations a cerebellovestibular connection was identified as the result of the orthograde transport of the injected enzyme. This material was kindly provided by Dr. Georgia Bishop at The Ohio State University. Hemicerebellectomies performed at 12 days of age revealed a pattern of degenerating cerebellovestibular fibers which closely resembled the orthograde labeling described above. At later ages there was a steady increase in the density of cerebellar projections to the vestibular nuclei, especially to the lateral vestibular nuclei bilaterally. At 16 days of age a few degenerating cerebellar axons were observed in the contralateral descending limb of the brachium conjunctivum. These fibers approached the level of the inferior olivary nucleus (IO) but did not appear to penetrate its nuclear subdivisions. However, by 20 days of age a marked accumulation of degenerating cerebellar axons appeared within IO. In several locations within IO these fibers ramified in and around groups of neurons in a pattern similar to that exhibited by adult degenerating cerebelloolivary axons. At the same time argyrophilic granules were observed in the reticular formation overlying the basilar pontine gray. In litters 26 days old degenerating preterminals were present in the appropriate portions of IO and the basilar pontine nuclei. The adult-like distribution of cerebellar projections to these two sites was established somewhat later. Supported by National Science Foundation Grant BNS 80-22312.

251.12 THE POSTNATAL DEVELOPMENT OF THE RAT PONTOPARAFLOCCULAR PROJECTION. C. E. Adams*, G. A. Mihailoff, J. K. Chapin Dept. of Cell Biology, The Univ. Tx. Hlth. Sci. Ctr., Dallas, Tx. 75235.

The ontogeny of motor behavior may depend in part upon the time course of initiation and maturation of transmission within the cerebro-ponto-cerebellar system. Ongoing investigations in this laboratory are concerned with aspects of the anatomical and physiological maturation of one component of the cerebro - ponto - cerebellar system, the projection from the visual cortex via the pons to the cerebellar paraflocculus. The aim of this study was to determine the time course of the postnatal development of the pontoparafloccular projection in the rat. Our previous anatomical studies demonstrated that fibers of the developing rat visual corticopontine system initially formed terminal projection fields on postnatal day 3. The subsequent maturation of these corticopontine terminal fields was characterized by a two-stage process. During the first stage (postnatal days 3-13), the terminal field distribution pattern was more diffuse than that observed in the adult. A gradual focusing or constricting of the terminal field distribution was seen during the second stage (postnatal days 13-20) which resulted in the formation of the adult projection pattern.

Unilateral injections of 0.2-0.3 microliters of the fluorescent dye nuclear yellow (1 mg/ microliter) were made into the paraflocculus of the cerebellum. Following a survival time which ranged from 19-23 hours, the animal was anesthetized and the brain fixed by intravascular perfusion of 4% phosphate buffered paraformaldehyde. The brainstem and cerebellum were then sectioned on a freezing microtome and the basilar pontine nuclei examined. On postnatal day 4, a crescent-shaped pattern of cell labeling was observed bilaterally within medial and lateral regions of the rostral one-half of the pons, with a contralateral predominance. Slightly more caudally, neuronal labeling was found to decrease and then disappear sequentially within: 1) the medial and lateral ipsilateral pontine zones, 2) the medial contralateral pontine zone, and 3) the lateral contralateral pontine zone. This pontoparafloccular projection pattern, seen also on postnatal days 7 and 9, corresponds to the pattern previously described for the adult. These observations indicate that the projection pattern of pontoparafloccular fibers is established in a mature configuration quite early in the course of postnatal development. In contrast, the visual corticopontine projection does not exhibit an adult pattern until postnatal day 20. The maturation of pontine neuron morphology is not adult - like until 24 days of age while synaptogenesis whithin the pontine neuropil is not completed until postnatal day 16. Supported by BNS 80-04853 and NS 18041.

251.13 PROJECTIONS OF CEREBELLAR NUCLEI IN THE PIGEON. J.J.A. Arends*, R.M.L. Faull* and H. Philip Zeigler, Biopsychology Program, Hunter College, City University of New York, 10021 and Dept. of Anatomy Univ. of Aukland, New Zealand.

A combination of autoradiographic and histochemical tracing procedures have been used to define the terminations of projections from the cerebellar nuclei and to clarify the topographic organization of the nuclei. In initial experiments, pressure injections of tritiated amino acids and horseradish peroxidase were made into the nuclei under stereotaxic control to define efferent pathways and their areas of termination. Large injections of horseradish peroxidase were then placed into the hindbrain, midbrain and forebrain and the organization of cerebellar inputs to these regions was analysed. In the final experiments, iontophoretic injections of HRP/lectin into selected target areas were used to clarify topographic organization within the nuclei. Cerebellar projections to the following structures were defined: Hindbrain: vestibular complex, inferior olive, reticular formation (paramedian, medial, lateral), pre-and perihypoglossal regions and intertrigeminal area: Midbrain: central grey, n. Darkschewitsch, n. interstitialis (Cajal), n. ruber and prerubral field: Diencephalon: n. spiriformis medialis, Stratum cellulare externum, n. intercalatus, n. dorsointermedius posterior thalami. Cerebellar projections are similar in many respects to those described for mammals. However, thalamic projections were quite restricted,perhaps reflecting the absence in birds of a thalamo-cortical motor system.

Supported by Grant MH-00836 and by Research Scientist Award MH-00320 and by Grant BNS 79-14238.

251.14 THE ORGANIZATION OF THE IPSILATERAL CEREBELLO-RETICULAR PATHWAY IN RAT AND ITS DIVERGENT COLLATERALIZATION. M. Bentivoglio*, M. Molinari* (SPON: European Neuroscience Association). Inst. of Neurology, Catholic University, Rome, Italy.

Since Cajal's description of an ipsilateral cerebellar descending pathway not much attention has been devoted in the literature to the organization of this system. However, it has been reported in anterograde tracing studies (e.g. Faull, R.L.M., J. Comp. Neurol., 178: 519, 1978) that the ipsilaterally descending fibers of the brachium conjunctivum terminate in the lateral parvocellular reticular formation. The present study was aimed at the identification of the cells of origin of the ipsilateral cerebello reticular system in the rat. Several retrograde tracers (horseradish peroxidase and fluorescent dyes) were injected in different cases in the lateral part of the medullary reticular formation. The injections were made obliquely under direct vision from the dorsal medullary surface. In all cases labeled cells were found in the ipsilateral deep cerebellar nuclei. The labeled cells were located in the dorsolateral hump (Dlh), a subdivision of the rat's cerebellar nuclei located dorsal to the border between lateral and interpositus nuclei, as well as in the lateral part of the interpositus. A few labeled cells were also seen in the lateral nucleus. Further anterograde tracing experiments are at present in progress in order to confirm that cerebellofugal fibers descend ipsilaterally to the medulla oblongata from the Dlh and the lateral part of the interpositus.

Moreover, a search was made for identifying whether the descending ipsilateral cerebello-reticular fibers are represented by axon collaterals of the ascending contralateral cerebello--thalamic pathway, or they take origin from separate cells. The fluorescent retrograde double labeling technique was employed in this study. Different combinations of fluorescent tracers were used. One tracer was injected in the lateral medullar reticular formation and the other was injected contralaterally in the thalamus. In these cases a high percentage of deep cerebellar cells labeled from the ipsilateral medullary injections were double labeled from the thalamic injections. These findings indicate that in rat deep cerebellar cells give off divergent axon collaterals ascending to the contralateral thalamus and descending to the ipsilateral medulla oblongata.

251.15 REEVALUATION OF THE NUCLEOCORTICAL PROJECTION OF THE CEREBELLUM. J. Courville, A. Legendre* and F. Faraco-Cantin*, Centre de recherche en Sciences Neurologiques, Université de Montréal, C.P. 6208, Montréal, H3C 3T8.

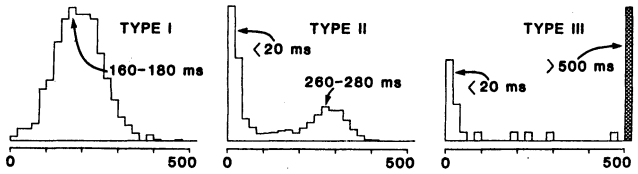
A projection from the cerebellar nuclei to the cerebellar cortex has been described in several species with methods of axoplasmic transport of aminoacids and horseradish peroxidase. It was concluded that this system terminates in the granular layer as mossy fibers and presents a topographical organization which resembles that of the corticonuclear projection. Anterograde transport of L-leucine of low (60 Ci/mmol) and high (147 Ci/mmol) specific activity was used to study the projection in the cat. With survival periods of one or two days, small (0.2 ul) injections of leucine of low or high specific activity in various region of the interposed and dentate nuclei demonstrate fibers of passage ascending in the white matter. Their density is low except for those directed to the flocculus and paraflocculus which are slightly more abundant. In the granular layer of the flocculus, small alignments and accumulations of silver grains produce a picture which resembles the terminal distribution of primary vestibular afferents to that region. In all other regions of the cerebellar cortex, only rare deposits of coarse grain alignments similar to those of the white matter are seen. At 6 and 8 days, the number of linear deposits in the white matter and granular layer of the various regions is slightly increased. Occasionally, small clusters of silver grains are observed in the granular layer. They could represent glomeruli or cross sections of the coarse fiber segments. There is a striking discrepancy between the number of labelled fragments in the white matter and the number of terminal structures in the cortex. At survival periods from 3 hours to 24 hours, the picture is however very different. Several grain clusters having the size of glomeruli are encountered in the granular layer after 6 hours. At 18 hours, they have already disappeared. These results confirm the presence of a nucleocortical projection terminating as mossy fibers. In all the cases utilized and with all survival periods, dense projections to the contralateral inferior olive have been observed. It is concluded that the fast transported proteins which label terminal structures have a rapid turnover. Material transported by slower axonal flow is more stable but mostly demonstrates fibers of passage and preterminal segments.

(Supported by the Canadian Medical Research Council).

252.1 DISCHARGE PATTERNS OF PUTAMEN NEURONS IN THE ABSENCE OF MOVEMENT IN THE MONKEY. J. Rajkowski*, M. Kimura* and E. V. Evarts. Laboratory of Neurophysiology, NIMH, Bethesda, Maryland 20205.

During quiescent waking most putamen cells are silent but some have tonic discharge. This abstract reports that tonically active putamen cells may be divided into two classes on the basis of their discharge patterns and the next abstract reports that these two classes are related to behavior in ways that are subtle but potentially important.

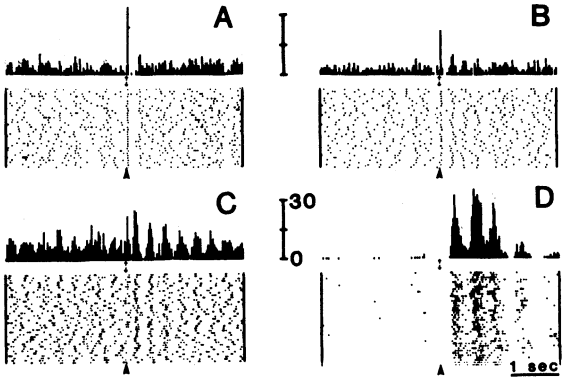
A microelectrode was advanced through the putamen during contralateral elbow flexion-extension movements that were periodically interrupted by the orofacial movements associated with consumption of a fruit juice reward. The 122 units upon which this report is based were initially picked up during limb and orofacial movements and then studied during a subsequent period of motor quiescence. 34 of these 122 neurons became virtually silent during absence of movement (average frequency less than 1 Hz). Such neurons (active with movement but otherwise silent) correspond to putamen neurons described by Crutcher and DeLong (1983). The activity of the 88 units that were not silent at rest fell into 3 categories, 2 of which had virtually continuous activity at between 3 and 7 Hz. In contrast, cells in the third category had relatively long periods of quiescence interrupted by occasional bursts. The first category of tonically discharging neurons (Type I) had a unimodal interspike interval distribution (IID) while the second category of tonic cells (Type II) exhibited a bimodal IID. Units that were for the most part silent but exhibited occasional bursts had IIDs with two modes, one of which was extremely short (corresponding to the intervals associated with the bursts) while the other was quite long, corresponding to the periods of silence. When units showing bursts and long periods of silence were carefully examined in relation to spontaneous movements occurring during periods that were otherwise quiescent, it was found that in fact their bursts were usually associated with slight spontaneous movements. IIDs for the 3 types are shown below with binwindths of 20 ms. There were no intervals greater than 500 msec for the Type I and Type II units, whereas the cross-hatched bar shows that pauses greater than 500 msec were common in Type III units.



252.2 EVENT-RELATED RESPONSES IN SPONTANEOUSLY ACTIVE PUTAMEN NEURONS. M. Kimura*, J. Rajkowski* and E. V. Evarts (SPON: J. C. Eberhart). Laboratory of Neurophysiology, NIMH, Bethesda, Maryland 20205

Tonically active putamen units were studied in 3 successive 40-trial blocks. SELF-PACED MOVEMENT (SPM): flexion-extension of elbow for juice reward followed by licking. FREE REWARD (FR): rewards delivered with arm position fixed; monkey promptly relaxed the arm with onset of FR. NO REWARD (NR): similar to FR except that the tube conveying the juice was occluded so the solenoid click was no longer followed by reward and licking extinguished after the first few solenoid clicks in the block of 40 NR clicks.

Tonically active units lacked intense discharge time-locked to movement, but type I tonic units often exhibited one or two impulses evoked at short-latency by the solenoid click preceding reward in SPM and FR, but not in NR, when click-triggered licks had extinguished. Type II units, (i.e., those with bimodal interspike interval distributions) rarely exhibited short-latency responses but did exhibit a synchronization of their tonic discharge following the solenoid click during SPM and FR. Synchronization also occurred in some type I units. Responses below are in SPM:



Solenoid click at arrow evoked short-latency responses in Type I unit in A, short-latency responses and synchronization in Type I unit in B, and synchronization alone in Type II unit in C. Movement-related activity time-locked to successive licks is shown in D for unit that was silent during arm movements at left of center and that then became intensely active as the monkey began to lick.

252.3 EFFECTS OF GLOBUS PALLIDUS LESIONS ON PAW REACHING BEHAVIOR IN RATS. U.E. Olazábal and J.S. Schneider. SUNY at Stony Brook, Stony Brook, N.Y. 11794 and Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

The globus pallidus (GP) has long been implicated in the control of arm movements. Findings from several studies suggest that the deficits in limb movements resulting from GP damage are not due to motor impairment, but are more complex in nature and involve sensorimotor processes. In an effort to understand the role of the GP in the forelimb reaching of rats, a fine-grained, high-speed film analysis of limb and postural movements was used.

Intact rats were trained to reach at various depths into a narrow tube with one forepaw to obtain food. A moveable piston positioned a food pellets 3mm to 23mm from the opening of the tube and thus determined the length of the paw reach. The wall of the experimental chamber to which the tube was attatched was opaque, hence eliminating the use of visual cues and making the response dependent on somatosensory and proprioceptive information. Body posture and limb movement were then photographed several times before and after lesioning. Paw reaching was rated for accuracy, duration, and posture. Normal rats easily reached and obtained pellets placed 10-20mm into the tube. Bilateral GP lesions caused obvious disruptions of this behavior. Typically, lesioned animals were inaccurate and unable to reach pellets placed within a few millimeters of the tube opening. Reach durations were also longer for the lesioned animals, particularly when the food pellets were positioned further from the tube opening. Also, the topography of the reaching movement in GP rats was characterized by a palm-sideways or palm-up posture, as opposed to the normal palm-down posture. This impairment contributed to the difficulties these lesioned animals had in grabbing and holding onto pellets. However, lesioned rats could pick up, manipulate, and eat pellets when they were visible and easily obtainable from a dish within the home cage. In addition, locomotion, grasping reflexes, and several other forelimb movements were not impaired by the GP lesions. Observations of gross postural changes showed that pre-lesion rats approached the tube staight on, tilted their heads sideways, and inserted their forelimb. After the GP lesions, various abnormalities in body position were noted-- a sideways approach to the tube accompanied by an exaggerated head tilt and body curvature.

These data indicate that disruptions of paw reaching behavior following GP damage are not simply due to a generalized forelimb motor deficit. Rather, basal ganglia damage interferes with the performance of organized limb movements whose succesful execution is dependent upon somesthetic and proprioceptive feedback. In addition, basal ganglia disruption of postural mechanisms contribute significantly to the observed forelimb movement deficits. (Supported by NINCDS Grant #16054).

252.4 DOPAMINE DEPLETION IN A NEOSTRIATAL SUBREGION DISRUPTS PERFORMANCE OF A SKILLED MOTOR TASK IN RATS. K. E. Sabol*, D. B. Neill*, S. A. Wages*, W. Church*, and J. B. Justice (SPON: K. Wallen). Depts. of Psychology and Chemistry, Emory University, Atlanta, GA 30322.

This experiment examined the role of specific subregions within the neostriatum in a skilled motor task requiring coordinated forelimb movements in the rat.

Food deprived rats were trained to reach with their preferred forelimb into a narrow slot to retrieve a 45 mg food pellet. Measures recorded were (a) successful attempts, (b) unsuccessful attempts, (c) individual reaches, and (d) the duration of each trial. Four trials/day were used; each trial included 10 pellets. After training, unilateral injections of 6-OHDA·HBr (8ug/2ul) were made into medial or lateral anterior neostriatum. Other rats received the ascorbic acid vehicle (0.1%) in either site. All injections were contralateral to the preferred paw. This surgery was performed using deep barbiturate anesthesia.

All rats were sacrificed approximately 6 wks. after surgery. DA depletion was determined by assaying (HPLC/EC) micropunches taken from medial and lateral neostriatum at different anterior-posterior levels.

Animals receiving lateral 6-OHDA injections showed a strong decrease in the number of successful attempts per total attempts (success ratio). This deficit was maintained for the 3 weeks of post surgical testing. Animals receiving medial 6-OHDA injections showed a smaller and transient decrease in the success ratio. Reach rate was impaired for both 6-OHDA groups but again the lateral deficit was stronger and longer lasting.

Qualitatively, the transient medial 6-OHDA induced deficit can be attributed to (a) rotational behavior or (b) a failure to initiate responding in a given trial. Rats with lateral 6-OHDA injections did not show consistent rotational behavior, and although they did show difficulty in initiating individual reaches, there was not an overall failure to respond. Their deficit seemed due to impaired coordination.

Dopamine assay data showed substantial DA depletion in the injected side with maximal depletion (15-20% of uninjected side) in the target striatal subregion. The deficit associated with the lateral 6-OHDA injected subjects appeared to be correlated with dopamine depletion in the lateral striatum at the level of the rostral pole of the globus pallidus.

These data support the hypothesis that skilled motor performance can be associated with a specific subregion of neostriatum.

252.5 SPEED IMPAIRMENT IN TRIGGERING AND EXECUTION OF LIMB MOVEMENT IN MONKEYS WITH LESION OF THE SUBSTANTIA NIGRA, F. Viallet*, D. Beaubaton, E. Trouche*, A. Nieoullon* and E. Legallet*. CNRS, INP, B.P. 71, 13277 Marseille Cedex 9, France.

Experiments were conducted on seven baboons with a view to establishing whether the substantia nigra (SN) is involved in the control of movement initiation and speed in monkeys. In a pointing task towards a fixed visual target, the latency, speed and accuracy of the baboons' pointing responses were determined from recordings of the reaction times, movement times and spatial errors. The results were then compared with the animals' performances after electrolytic or 6-OHDA SN lesions. The experimental conditions were varied with regard to either the spatial characteristics of the limb trajectories or the use of open or closed loop visual cues. Furthermore, the effects of electrolytic lesion, entailing destruction of the dopaminergic neurons, were compared with the specific effects of lesions produced with 6-OHDA.

After electrolytic SN lesions, the animals showed akinesia of the whole controlateral half of the body, with semi-flexion of the limbs for one to two weeks. They then took three months to recover their previous ability to perform the pointing task, initially showing increased latency and considerably decreased velocity in the contralateral limb, although the spatial accuracy was almost unaffected.

Animals with 6-OHDA lesions showed only mild contralateral akinesia which took less than 48 hours to recover. Nevertheless, they also exhibited a considerably decreased velocity in the contralateral limb whereas the accuracy and latency of the pointing response were not affected. Moreover, about 20 days after the 6-OHDA injection, an increase in movement latency was noted, which showed no tendency to recover.

When visual afferents were missing considerable impairment of movement occurred, and a comparison between visual open loop and closed loop performances revealed a significant interaction between the conditions of vision and SN lesion.

It thus emerges from these data that the SN is certainly involved in the control of movement initiation and speed. The results moreover clearly show that the role played by the dopaminergic system in the triggering of movement should not be overlooked. Lastly, as in human Parkinsonian subjects, visual information was used by these animals to compensate for motor deficits.

Investigations on the feedforward and feedback control of motor processes in primates should therefore take the probable involvement of the SN into account.

252.6 EFFECTS OF CHRONIC HALOPERIDOL ADMINISTRATION ON NEURONAL SENSITIVITY TO IONTOPHORESED GABA WITHIN RAT GLOBUS PALLIDUS, J. M. Frey, M. K. Ticku, and R. D. Huffman, Department of Pharmacology, Division of Neuropharmacology, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284.

Recent evidence suggests that chronically administered haloperidol results in an increase in GABA receptor binding sites in the substantia nigra (Gale, K., Nature 283:569,570,1980), possibly as a result of a decrease in the activity of the GABAergic strionigral system. The globus pallidus also receives a major GABAergic projection from the striatum; therefore, we decided to look at possible changes in GABA responsiveness of single pallidal neurons in rats that had been treated chronically with haloperidol. The effects of microiontophoretically applied GABA were assessed and compared on single pallidal neurons in control rats and in rats that had been fed haloperidol for 30 days (9.8±1.4 mg/kg) and then withdrawn from the haloperidol diet for 2 days. GABA binding in the substantia nigra is significantly elevated for at least 4 days after discontinuation of the chronic haloperidol feeding (Huffman, R.D. and Ticku, M.K.,Pharmac. Biochem. Behav. 18, 1983,In Press). Male Sprague-Dawley rats (250-300g) were anesthetized by the intraperitoneal injection of chloral hydrate (350 mg/kg) and a tracheotomy and jugular cannulation were performed. A left craniectomy was performed to expose the cortex overlying the globus pallidus. Extracellular single unit activity was monitored utilizing standard extracellular microelectrode recording techniques. GABA was applied by microiontophoresis from 5-barrel glass micropipettes with total tip diameters of 14-25µm. Drug concentrations utilized were as follows: GABA (5 and 10 mM in 0.2M NaCl, pH 3.8-4.0), dopamine (0.2M, pH 4.0), NaCl (3M for recording and 1M for current control). Electrodes were filled by centrifugation and utilized within a short time thereafter. In order to quantitate the sensitivity of GP neurons to iontophoresed GABA, EC_{50} values were estimated from dose-response curves compiled from the results of the systematic application of various ejection currents for two consecutive 20 sec recording periods. GABA produced profound inhibition of GP cell firing in both control and haloperidol-treated rats, but the haloperidol-treated group showed a statistically significant increase in GABA responsiveness with an average EC_{50} value 2.5 times less than controls (33.2±5.2 versus 84.4±12.2, df=34, $p<0.01$). Preliminary binding studies of GP tissue punched from 800µm frozen tissue sections revealed a 35% increase in the specific {H^3}-GABA binding within the treatment group when equilibrium GABA binding was measured using 4 nM{^{3}H}-GABA ($p<.07$). These data support the hypothesis that increases in {^{3}H}-GABA binding within the GP after chronic haloperidol treatment are accompanied by an increase in neuronal responsiveness to GABA.

252.7 EFFECTS OF PRENATAL HALOPERIDOL ON RECEPTORS IN THE DEVELOPING RAT STRIATUM: OPPOSITE CHANGES IN NALOXONE AND SPIPERONE BINDING. Sandra Moon Edley. Laboratory of Neurophysiology, National Institute of Mental Health, Bethesda, Maryland 20205.

A number of studies have shown decreased opiate binding to the the rat striatum after mechanical or chemical destruction of the nigrostriatal tract. Both of these approaches destroy not only a structural element of the striatum, namely, the nigral terminals, but also eliminate a chemical element, dopamine, and its regulatory effects. To further elucidate the interactions of the opiate and dopamine systems, dopamine receptors were blocked by haloperidol during an early dynamic period of striatal development.

Pregnant rats were anesthetized with ether on embryonic day 15 (E15), and 7-day osmotic minipumps filled with haloperidol (1 mg/day) or saline (0.9%) were implanted subcutaneously. Pups were decapitated on postnatal day 0 (P0) or 16 (P16). The frozen brains were cut in a cryostat. Some sections were processed immediately for dopamine fluorescence using a glyoxylic acid method. Others were incubated in either ^{3}H-naloxone, ^{3}H-spiperone or ^{3}H-muscimol to mark opiate, dopamine and GABA receptors, respectively, and apposed to ^{3}H-sensitive film. The film images were computer-analyzed to quantify ligand binding densities in the striatum.

Binding in tissue from animals treated with saline did not differ from untreated animals. At P0 the emerging heterogeneous pattern of naloxone-labeled opiate receptors is not altered by prenatal haloperidol. However, the density of opiate binding is reduced to about 70% of normal levels. This depressant effect of haloperidol was not general: in the same brains ^{3}H-spiperone binding, showing the normal modest high-lateral, low-medial gradient, was elevated by as much as 22%. The ^{3}H-muscimol distribution was patchy and unchanged.

By P16 naloxone-labeled receptors assumed the adult pattern of dense patches and subcallosal streak on a lightly labeled matrix. In the haloperidol-treated cases the adult pattern of naloxone labeling was acquired, but the density was slightly reduced. However, the partial recovery lagged in the patch (and streak) areas as compared to the non-patch zones. ^{3}H-spiperone, still showing a lateral-medial gradient, remained slightly elevated. The homogeneous muscimol binding was normal.

Dopamine, as demonstrated by fluorescence histochemistry, was present in the striatum, thereby discounting the possibility that nigral neurons were destroyed by the chronic haloperidol treatment. The finding of reduced naloxone binding after haloperidol treatment does not resolve whether a subset of opiate receptors is located on nigral endings, but it does suggest that striatal naloxone binding is modifiable through a mechanism involving dopamine receptor function.

252.8 OPPOSITE DIRECTIONS OF CIRCLING PRODUCED BY MEDIAL AND LATERAL SUBSTANTIA NIGRA LESIONS: ROLE OF MIDBRAIN. F.J. Vaccarino* and K.B.J. Franklin. (SPON: L.A. Switzman). Psychology Dept., McGill Univ., Montreal, Que., Canada H3A 1B1.

It is well known that unilateral stimulation or lesions of the nigrostriatal dopamine pathway cause animals to circle away from the more active nigrostriatal system. Thus, after unilateral substantia nigra (SN) lesions the indirect stimulant amphetamine causes rats to circle toward the lesioned side while apomorphine, acting on supersensitive DA receptors, produces circling contraversive to the lesioned side. Recently we have reported that when lesions or stimulation are restricted to the lateral portion of the SN rats circle towards the stimulated or more active side (Vaccarino and Franklin, Behav. Brain Res., 5:281, 1982; Vaccarino and Franklin, Pharmac. Biochem. Behav., 17:431, 1982). These findings indicate that the lateral portion of the SN acts in opposition to the more medial portion.

It has been shown that the main output pathway involved in nigrostriatal circling runs from the striatum to the pars reticulata of the SN and thence to the superior colliculus (SC) and midbrain reticular formation (MRF). To see if the same pathways were involved in the circling elicited from the lateral SN the present study examined the effects of lesions in the MRF and SC on circling following lesions of either medial or lateral SN. Consistent with earlier reports, Experiment 1 showed that unilateral SC lesions produced ipsiversive circling. Rats with combined medial SN and SC lesions (in the same hemisphere) showed no more ipsiversive circling than rats with SC lesions alone. In contrast to the non-additivity of the latter effects, the contraversive circling produced by lateral SN lesions was additive with the ipsiversive circling produced by SC lesions. Using the same design experiment 2 showed that neither the ipsiversive circling produced by medial SN lesions nor the contraversive circling produced by lateral SN lesions was additive with the ipsiversive circling produced by MRF lesions. Amphetamine dose-dependently increased circling in all rats without affecting direction.

Experiment 1 suggests that while the SC may be an output for medial SN derived circling it is not an output for the opposing lateral SN derived circling. Experiment 2 suggests that the MRF may be an output for both medial and lateral SN derived circling. This implies that medial and lateral nigrostriatal DA activity may modulate MRF activity in opposite directions.

(Supported by NSERC Grant A6303 and The Parkinson Foundation of Canada.)

252.9 CONDITIONED TURNING: EFFECTS OF UNILATERAL 6-OHDA LESIONS AND SIDE OF SPONTANEOUS TURNING PREFERENCE, S. B. Dunnett and A. Bjorklund. Department of Experimental Psychology, Cambridge, UK and Department of Histology, Lund, Sweden.

Unlesioned rats can be trained to rotate selectively in one direction and such turning is reported to be associated with changes in dopamine and DOPAC levels in the contralateral neostriatum (Yamamoto et al. Life Sci. 30:2155 1982).

Experiment 1. Fourteen rats under 23 hour water deprivation were trained to rotate in a hemispheric bowl in one direction for 0.1µl sugar water reinforcement, delivered to a nozzle in the wall of the bowl. After 9 days training, all rats received a unilateral lesion under barbiturate anaesthesia by injection of 8µg 6-OHDA in 4µl ascorbate-saline into the ascending dopamine mesotelencephalic bundle. The lesion was made on the same ("ipsilateral" group) or the opposite ("contralateral" group) side to the direction of initial training. Two weeks later, training continued over 4 days to turn in the same direction as previously. "Ipsilateral" rats continued to show high rates of selective turning in the reinforced direction whereas "contralateral" rats showed equal turning in both directions (group x direction, $F(1,12)=18.93$, $p<0.01$). The reinforced direction of turning was then reversed for a further 6 days "Ipsilateral" rats were unable to learn to rotate in the now reinforced direction contralateral to their lesion whereas the "contralateral" rats rapidly learned to turn selectively towards the now reinforced side (group x direction, $F(1,12)=5.10$, $p<0.05$).

Experiment II. Twenty-four unlesioned rats were tested for rotation under 5mg/kg amphetamine in automated rotometers. Three groups of 3 rats were selected which showed either >100 net right turns, >100 net left turns, or no turning bias over the 90 min test. These 9 rats were all trained to turn to the right for sugar-water reinforcement, initially under continuous reinforcement (each full turn to the right reinforced), then under fixed ratio 2, 3 and 4 schedules for 3 days each. All rats rapidly learned to turn selectively in the reinforced direction, and no differences were detectable between the 3 groups at any stage of testing.

Conclusions. Spontaneous side asymmetries in intact rats do not influence their ability to learn to turn selectively in one direction. 6-OHDA lesions producing >95% forebrain dopamine depletions abolish the rats' ability to learn to rotate selectively contralateral to the lesion. Neither motivation nor primary motor impairments can account for this pattern of results, which suggest rather that the lesion disrupted the ability to initiate contralateral turns.

252.10 BASAL GANGLIA MEDIATION OF CHEMICAL-INDUCED HYPERKINESIA IN RATS. J.S. Schneider, Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

Daily administration (7-10 days) of 3-3 iminodipropionitrile (IDPN) results in a complex hyperkinetic syndrome in rats, characterized by a spontaneous and permanent choreo-athetoid cervical dyskinesia and bursts of circling and locomotor activity. The cervical dyskinesia consists primarily of large amplitude vertical choreotic movements and to a lesser extent, lateral athetoid movements. While IDPN produced a motor disorder in all rats tested, there was much variability as to the severity of the disorder. While circling (both sides) and increased locomotor behavior were observed in some rats, others appeared somnolent and displayed low levels of locomotor behavior. Cervical dyskinesias were observed both when rats were moving and when they were stationary. In rats with prominent spontaneous head movements, stress greatly increased the frequency (and often the magnitude) of these movements. All rats had some degree of postural disorder consisting of body tilt to one side with forelimbs extended.

Five rats with severe spontaneous head movements were given bilateral globus pallidus lesions. These lesions were effective, in all cases, in permanently eliminating or greatly reducing the frequency and magnitude of both spontaneous and stress-induced head movements (mean post-lesion decrease in spontaneous movements of 95%).

Two rats with prominent spontaneous movements were given large bilateral ablations of sensory and motor cortices. These lesions had minimal effects on either the frequency or magnitude of head movements.

Another four rats with distinct head movements were given bilateral trigeminal ganglion lesions. Removal of most of the sensory inputs from the face was successful in reducing the frequency and magnitude of spontaneous head movements (mean 75% decrease) but had little or no effect on stress- or amphetamine induced head movements.

These results suggest that this chemically-induced motor disorder has a significant basal ganglíar component, since removal of basal ganglia outputs virtually eliminates the abnormal movements. It is suggested that descending influences from the pallidum are important for mediation of this disorder since sensori-motor cortex lesions are ineffective in attenuating the abnormal movements. Lastly, the reduction in spontaneous movements following loss of sensory inputs is consistent with the idea that the basal ganglia may play a significant role in the gating of sensory information to brainstem and cervical motor areas (Schneider et al., Exp. Neurology 77: 534-543 and Olazabal and Lidsky, Soc. Neurosci. Abst. 8: 960, 1982).

Supported by USPHS HD 07032.

252.11 SIMULTANEOUS CATALEPSY AND APOMORPHINE-INDUCED STEREOTYPIC BEHAVIOR IN MICE. D.M. Yurek and P.K. Randall (Spon. S. Erlich) Dept. of Physiology & Biophysics, USC Sch. of Med., Andrus Gerontology Ctr., L.A., CA 90089.

The classical neuroleptics, haloperidol (HAL), chlorpromazine (CPZ) and spiperone (SPIP) effectively block apomorphine (APO)-induced stereotypic behavior in mice when injected intraventricularly at low doses producing little or no catalepsy. Domperidone (DOM) does not cross the blood-brain barrier and sulpiride (SULP) has limited access to the CNS. When administered intraventricularly both produce not only a highly cataleptic response but also a simultaneous augmentation rather than diminution of stereotypic behavior, especially gnawing.

Both C57BL/6J and BALB/cJ mice were surgically implanted with bilateral cannula allowing for direct injection of neuroleptics into the lateral ventricles. HAL (50 ug), CPZ (50 ug), SPIP (10 or 2 ug), DOM (50 ug), and SULP (2 or 0.5 ug) were injected intraventricularly (inj. vol. = 1.0 ul in each ventricle) followed 40 minutes later by a systemic injection of APO (2.0 mg/kg) to test for stereotypic behavior. Catalepsy tests were given 5 minutes prior to, and 10, 20, 45 and 75 minutes post APO. HAL, CPZ, and SPIP produced catalepsy at all times and blocked the expression of stereotypic behavior. High doses of SULP and DOM produced similar results. Low doses of SULP and DOM induced only a slight cataleptic response pre-APO. However, a few minutes after the APO injection we observed a coexisting state of catalepsy and stereotypic behavior in both strains. In addition, at high doses, SULP mice exhibited immediate stereotypic gnawing upon external sensory stimulation.

The behavior of the low dose SULP mice post-APO can be characterized as initially showing augmented stereotypic behavior over the controls and an unusual, persistent licking behavior that was not observed with any other treatments. During the early post-APO catalepsy tests both the low dose SULP and DOM mice maintained a rigid cataleptic posture while simultaneously gnawing and licking at the floor.

Our observations suggest that catalepsy and stereotypic behavior do not lie on opposite ends of a continuum. Rather, the specificity SULP and DOM have for D-2 receptors indicate that there possibly exists a subset of D-2 receptors that are not blocked by SULP or DOM and are specific for stereotypic behavior. Thus, both cataleptic and stereotypic behavior are elicited simultaneously. The augmented stereotypic behavior was marked with an unusual gnawing and licking behavior.

These results may be related to the clinical observations of coexistent tardive dyskinesia and drug-induced Parkinsonism in individual patients.

252.12 QUANTIFICATION OF BEHAVIORAL SUPERSENSITIVITY IN THE C57BL/6J MOUSE. R.J. Mandel* and P.K. Randall* (Spon. D.F.Lindsley)USC Sch. of Med, Depts. of Psych. and Physiology and Biophys. , and Andrus Gerontology Ctr. L.A., CA. 90089

Until recently, the rotational paradigm for measuring behavioral supersensitivity to DA agonists has not been entirely meaningful due to the lack of a relevant control group for comparison. Marshall and Ungerstedt (Eur. J. Pharm. 41:361-367,1977) first described the use of a lesion which completely blocked the behavioral output of one striatum. This enables one to obtain turning data which theoretically is caused by the behavioral outflow of one nonsupersensitive striatum in the rat. Their lesion is thought to be an interruption of the striatonigral projectons and is located in the ventral portion of the crus cerebri in rats. The present study has used this method to quantify the behavioral supersensitivity in the C57BL/6J mouse.

The lesion placement that was found to be most effective in the C57 corresponds closely to that found in the rat. A discrete electrolytic lesion of the ventral internal capsule (-1.6 AP, +2.0 Lat, -4.5 DV from bregma) produces reliable and marked ipsiversive turning to 2 mg/kg amphetamine. A repeated measures design was undertaken to determine the dose response curve for apomorphine. These same animals were then given 6-OHDA lesions in the opposite striatum and maintained for 21 days to allow supersensitivity to develop in the striatum with an intact output. These supersensitive mice were also treated in the repeated measures design to obtain another dose response curve for apomorphine for the supersensitive preparation.

The equation for the regression line for the pre-6-OHDA lesion dose response is $Y=42X+26$ with $r=.992$. The equation for the regression line for the post-6-OHDA lesion dose response is $Y=34X+149$ with $r=.985$. These data indicate nearly parallel dose response curves with a 32 fold increase in sensitivity.

The parallel dose response curves in a within animal design indicate a true shift of the dose response curve with no contamination with increased amplitude of response. This method is an important improvement in the rotational paradigm because it allows comparison of increases in sensitivity and not actual numbers of rotations. Therefore many organismic variables such as inherent differences in baseline activity levels or differential reactivity to drugs between different strains or ages of animals are controlled for and comparisons of disparate groups are meaningful. Taken together, these advantages make this technique a powerful tool for studying the parameters of behavioral supersensitivity in inbred mouse strains.

252.13 THE EFFECTS OF INTRASTRIATAL AND CORTICAL INJECTIONS OF SCOPOLAMINE ON POSTURAL DEVIATION AND REARING BEHAVIOR IN RATS. L.K.Chambers and C.Van Hartesveldt. Dept. of Psychology and the Center for Neurobiological Sciences, Univ. of Florida, Gainesville, FL 32611.

Intrastriatal injections of the anticholinergic drug scopolamine (SCOP) has been reported to elicit contralateral postural deviation and stereotyped rearing in separate experiments. We designed the present research to investigate the dose-response characteristics of both behaviors and to test the behavioral effects of the drug in the neocortex above the striatum.

Male Long-Evans hooded rats were implanted bilaterally with permanent guide cannulae (21 GA), so that the 27 GA injection cannulae were located in the anterior caudate-putamen (CPU) or rostral neocortex. Animals were unilaterally injected with either the vehicle (VEH) or one of three doses of (SCOP) 20, 30 or 40µg/.25µl buffer. After injection, animals were placed in a circular clear plexiglass chamber and monitored for postural deviation and number of rears. The cumulative scores for each 5-minute block of the 30 minute observation session were recorded. For statistical analysis, a dominant direction score for deviation was obtained by subtracting ipsilateral scores from the contralateral scores. The data were then analyzed with a factorial ANOVA for repeated measures, using the absolute difference scores between VEH and SCOP.

Animals with injections of SCOP into the anterior CPU showed consistent contralateral postural deviation with no clear dose related differences. Injections of SCOP into neocortex did not elicit contralateral postural deviation.

Unilateral CPU injections of SCOP produced significant increases of rearing which were dose related, but neocortical injections were also effective. There was a trend for neocortical injections to produce more rearing with a shorter latency of onset than CPU injections. Closer examination of exact neocortical injection sites, along the anterior-posterior dimension, indicated that the closer the site to the region dorsal to the CPU the shorter the latency to onset of rearing. These results suggest that while SCOP-induced contralateral deviation is localized in the CPU, SCOP-induced rearing may be localized in the neocortex.

252.14 PERCEPTUAL MOTOR DYSFUNCTION IN PARKINSON'S DISEASE IS RELATED TO A DISTURBANCE IN THE HIGHER-ORDER CONTROL OF MOVEMENT. Y. Stern*, R. Mayeux*, J. Rosen*, and L. J. Cote, Neurological Inst., Columbia Univ. College of Physicians & Surgeons, and City Univ. of the City of New York, New York 10032.

Perceptual motor dysfunction is the most common form of intellectual impairment in Parkinson's disease (PD). This behavior may be related to a disturbance in higher-order control of movement, particularly predictive or sequential movements. Fourteen parkinsonian patients without dementia or depression and 9 age-matched controls used a stylus to trace paths, including a horizontal line and a sawtooth pattern, presented on a vertical screen. After tracing each of these, subjects were presented with degraded paths: the line was degraded into two endpoints and the sawtooth path was degraded by eliminating a straight portion or one or two angles. Subjects filled in the missing portions. Previously we rated videotaped tracing performance (Stern et al. JNNP 1983;46:145-51), but in this study digitizing apparatus quantified tracing error (deviation from the path) and velocity during tracing. Subjects also completed a construction test and a measure of general intellectual function, and the severity of patients' parkinsonian symptoms was rated. Patients' tracing error was greater than controls' on all paths despite comparable tracing velocity in the two groups. All subjects performed more poorly as the paths were successively degraded, but patients' accuracy in tracing deteriorated more rapidly than controls'. Tracing error on the degraded paths correlated only with patients' performance on the construction task and was not related to the severity of their disease. Errors in the controls related only to general intellectual ability. This study suggests that the patients have a disturbance in the higher-order control of movement that is not related to the typical motor manifestations of PD and has a similar basis as poor performance on construction and other perceptual motor tasks. Both performance deficits represent an inability to formulate or act upon internal representations of space and are a consequence of basal ganglia degeneration.

This work was supported by the Parkinson's Disease Foundation.

252.15 BRAINSTEM DYSFUNCTION IN DYSTONIA MUSCULORUM DEFORMANS SUGGESTED BY POLYSOMNOGRAPHIC FINDINGS. W.R.Jankel, E.Niedermeyer*, M.Graf*, R.Allen*, and M.Kalsher*. Clin.Neurophysiol.Lab., Johns Hopkins Univ.Sch.of Med. and Hospital, Baltimore, MD 21205.

Dystonia musculorum deformans (DMD) is a progressive, heterogeneous disorder of unknown pathophysiology with three hereditary forms and appearing as a symptom in 20 other diseases. Intelligence is unaffected. Based on reports of relief from dystonia during sleep, the nocturnal EEG, EOG, and EMG were studied in nine patients with DMD and nine healthy controls.

Gold-plated silver cup electrodes were placed over frontal, central, and occipital regions in accord with the International 10-20 System. A standard bipolar montage was used for the recordings and EEG records were scored independently using the criteria of Rechtschaffen and Kales.

All of the dystonia patients were found to be poor sleepers. However, the sleep of patients in advanced stages of DMD revealed an EEG pattern which was characterized by pronounced, high amplitude (75-100µV) sleep spindles which were continuous for all of stage 2 and portions of stage 3 NREM sleep. Other sleep parameters including latency to sleep, per cent time in REM sleep, number of REM periods, and number of awakenings were progressively abnormal as the dystonia advanced.

Gibbs and Gibbs(1964) reported an exaggeration of normal sleep spindles which they called "extreme spindles" in young, cerebral palsied and mentally retarded children. These spindles were found most often in children less than 5 years of age and never in children older than 12. They suggested that the "extreme spindle" activity was associated with extrapyramidal dysfunction since cerebral palsy alone was insufficient to produce the spindles.

In normal healthy persons, sleep spindles become less frequent and diminish in amplitude with advancing age. The spindle activity of advanced DMD cases presents a stark contrast to this normal pattern. It differs from the "extreme spindle" pattern by a later age of onset (12-56yrs.of age), is not accompanied by retardation, and variations in spindle frequency within patients, is absent.

Successful treatments, in addition to relieving the clinical symptomatology, also normalize the sleep of these patients and reduce the amplitude of the sleep spindles. This may underscore the clinical significance of these sleep spindles as an index of the dystonia process, and further, suggest brainstem involvement in this disorder.

The elucidation of a sleep disorder which parallels advancing dystonia suggests that polysomnography may be an important adjunct to biochemical studies of dystonia and other presumed basal ganglia disorders.

253.1 DISTRIBUTION OF ACETYLCHOLINESTERASE IN THE MEDIAL AND VENTRAL PREFRONTAL CORTEX OF THE RHESUS MONKEY. H. Barbas, and D. N. Pandya. SAR, Boston Univ., and V.A. Med. Center, Bedford, MA.

Architectonic studies have shown that the prefrontal cortex contains two regions with a rudimentary laminar organization. One of these regions is situated on the medial surface of the frontal lobe at the rostral tip of the cingulate sulcus, and the other is located in the caudal orbitofrontal cortex. Each of these regions contains a bilaminar agranular cortex, (designated periallocortex), which is bounded by regions with an incipient isocortical type of laminar organization (called proisocortex). From each of these periallo-proisocortical (pAll-Pro) areas a stepwise laminar differentiation can be traced within the prefrontal cortex (Barbas and Pandya, 1982).

In addition to their distinct cytoarchitecture, as observed in Nissl stained sections, the frontal pAll-Pro areas can be distinguished from neighboring regions in material reacted for the visualization of acetylcholinesterase (AChE). Compared to dorsolateral frontal isocortices, the pAll-Pro areas contain heavier AChE activity, concentrated in distinct bands in layer I, and in the deep layers. Whether the heavy AChE activity observed in pAll-Pro areas is due predominantly to intrinsic AChE-positive neurons and their processes or to afferent projections, is not known. However, when AChE activity within fibers is suppressed by diisofluorophosphate pretreatment of the tissue, a similar number of AChE-positive neurons can be seen in layer VI in both pAll-Pro areas and dorsolateral isocortices. This suggests that the heavy AChE activity in the pAll-Pro areas in normal tissue is mainly due to AChE-containing afferent projections to the prefrontal cortex. This observation may be significant because AChE-rich pathways are potentially cholinergic and have been implicated in mnemonic processes.

In a previous study it was shown that the prefrontal pAll-Pro areas have widespread connections within the prefrontal cortex, compared to restricted intrinsic connections of prefrontal isocortices. This suggests that the prefrontal pAll-Pro areas may exert a broad influence on the prefrontal cortex.

On the basis of the above observations it is possible that disruption of putative cholinergic pathways in some of the degenerative neurologic disorders may partially deafferent the pAll-Pro areas, and may thus affect the normal functioning of the rest of the prefrontal cortex.

Supported by Seed Grant GRS-691 from Boston University, and by E.N.R.M. Veterans Hospital, Bedford, MA.

253.2 ALPHA AND BETA RECEPTOR MEDIATED EFFECTS ON PURKINJE CELL SPONTANEOUS ACTIVITY IN THE IN VITRO RAT CEREBELLAR SLICE, A. S. Basile and T. V. Dunwiddie. Dept. Pharmacology, Univ. Colo. Health Sciences Center, Denver, CO 80262.

Superfusion of Purkinje neurons in the in vitro rat cerebellar slice with norepinephrine caused dose-dependent increases and/or decreases in spontaneous Purkinje neuron firing rates. The excitation evoked by low concentrations of norepinephrine (0.5-10 uM) and by all concentrations tested of the beta receptor agonist isoproterenol (0.1-5 uM) were reduced by timolol, a beta receptor antagonist. Perfusion with higher concentrations of norepinephrine (25-100 uM) or with the alpha receptor agonist clonidine (0.5-50 uM) caused a depression of Purkinje neuron spontaneous activity. This inhibitory response was blocked by the alpha receptor antagonist phentolamine. The alpha-one selective agonist phenylephrine had no effect on spontaneous firing rates at concentrations up to 100 uM. All of these responses appeared to be due to a direct action on the Purkinje neurons, because neither the excitation nor the depression of Purkinje neuron activity caused by norepinephrine was substantially altered in a medium with reduced calcium and increased magnesium concentrations, which has been found to block synaptic transmission in vitro. The amplitude of excitatory responses appeared to be dependent upon the potassium concentration of the medium, such that the mean excitatory response elicited by 500 nM isoproterenol (218% above control at 5.2 mM K^+) was reduced to 3% at 3 mM K^+. Clonidine induced depressions were not significantly altered by the changes in the concentrations of potassium in the medium. However, it was not possible to determine if these were direct effects of potassium or due to changes in basal firing rates produced by changes in the potassium concentration of the medium. In summary, under these in vitro conditions, norepinephrine appears to increase the firing rate of Purkinje neurons via an interaction with beta adrenergic receptors, while norepinephrine induced depressions may be mediated by alpha adrenergic receptor interactions; both receptors appear to be located on the Purkinje neuron itself. Preliminary evidence suggests that potassium may be directly or indirectly involved in the excitatory responses elicited by norepinephrine.

This work was supported by DA 02702 and VA 394463116-01 to T.V.D. and 5T32GM7635 to A.S.B.

253.3 THE EFFECT OF DESMETHYLIMIPRAMINE (DMI) AND RESERPINE ON PROTEINS FROM THE PARIETAL CORTEX AND THE HIPPOCAMPUS AS ASSESSED BY TWO-DIMENSIONAL GEL ELECTROPHORESIS (2DE). William E. Heydorn, G. Joseph Creed* and David M. Jacobowitz. Lab. of Clinical Science, NIMH, Bethesda, MD 20205.

Using 2DE, we have recently compiled an atlas of proteins from 25 discrete regions of the rat brain. Each protein was analyzed for molecular weight, isoelectric point (pI) and relative quantity, and assigned a permanent indexing number. Using this map, we now report on the effect of the tricyclic antidepressant DMI and the neurotransmitter depleting agent reserpine on proteins in the parietal cortex and hippocampus. In the first experiment, rats received either DMI 10 mg/kg IP twice daily for 3 weeks or a single 10 mg/kg dose of the antidepressant. Appropriate control groups received 0.9% NaCl. Rats were killed by decapitation 24 hours after their last dose, and tissue samples of parietal cortex and hippocampus were obtained by microdissection. Proteins were separated by 2DE, visualized using a silver stain, and quantitated by computerized scanning densitometry. Of the 125 proteins analyzed, repeated DMI administration produced a reduction in the concentration of two proteins in both the parietal cortex and the hippocampus. The first, identified on our maps as protein #10 (molecular weight 57,000 daltons, pI 6.2) was reduced by 65% ($p<0.005$) in the parietal cortex and 63% ($p<0.001$) in the hippocampus by chronic DMI administration, while the second, identified as protein #11 (molecular weight 57,000 daltons, pI 6.3) was reduced 41% ($p<0.005$) in the parietal cortex and 52% ($p<0.001$) in the hippocampus. In contrast, protein #41 (molecular weight 28,000 daltons, pI 5.9) was elevated by about 22% in both regions as a result of chronic DMI administration. In all three cases, acute drug admistration was without effect.

In a second experiment, rats were treated either acutely or repeatedly with reserpine. One group of rats received 2.5 mg/kg of reserpine once daily for 3 days and was killed on day 4. Another group of rats received a single 2.5 mg/kg dose of reserpine and was killed 30 minutes later. Control animals received 0.9% NaCl. In the hippocampus, protein #10 was increased 36% ($p<0.05$), protein #11 was increased 44% ($p<0.025$) and protein #41 was reduced 28% ($p<0.001$) in rats treated repeatedly with reserpine. In contrast, chronic reserpine administration had no effect on any of these three proteins in the parietal cortex. In all three cases, acute drug treatment was without effect. These results indicate that specific proteins within the rat brain can be altered by the chronic administration of agents affecting noradrenergic reactivity, and are of interest in view of the known effects of these drugs on neurotransmitter receptors. In addition, there appears to be some regional specificity as to the effect of these drugs among different neuroanatomical areas of the rat brain.

253.4 THE SUBSTRATE FOR SELF-STIMULATION OF THE PREFRONTAL CORTEX IN THE RAT: STRENGTH-DURATION CHARACTERISTICS. S. Schenk* and P. Milner*, Dept. Psychol., McGill University, Montreal, Quebec H3A 1B1, and P. Shizgal, Center for Studies in Behavioural Neurobiology, Concordia University, Montreal, Quebec, H3G 1M8.

The rewarding effects of stimulating the prefrontal cortex (PFC) and the lateral hypothalamus (LH) appear to be mediated by different populations of directly stimulated cells. In the present study we attempted to further differentiate these two populations by obtaining estimates of their strength-duration characteristics. These estimates may prove useful in distinguishing the neurons responsible for the rewarding effects from other neurons activated by the electrodes and hence, may expedite the identification of the reward-related cells by electrophysiological means.

Strength-duration functions were obtained from two PFC and two LH sites by determining pairs of currents and pulse durations that sufficed to maintain a half-maximal rate of lever pressing. The duration of the cathodal, constant current pulses ranged from 0.5 to 15.0 msec. Throughout the experiment train duration and pulse frequency were fixed at 0.5 sec and 25 Hz, respectively.

In order to standardize the results across placements, hyperbolae were fit to the strength-duration data, and the current (I) values were then divided by the rheobase (r). A three-way ANOVA (subject x duration x site), performed on the logarithms of these I/r values, yielded a significant interaction between pulse duration and site. This indicates that the strength-duration curves for the PFC and LH sites differ in shape. Indeed, the chronaxies of the PFC curves were 0.98 and 1.25 msec while the chronaxies of the LH curves were 1.59 and 1.90 msec.

Matthews (J.c.p.P., 1977, 91, 858-874.) has noted that the chronaxies of behaviorally derived strength-duration curves for the LH reward substrate are much longer than most electrophysiologically derived strength-duration curves for single central neurons. He proposed that the LH reward substrate must include some elements with unusually long chronaxies or neurons that fire repetitively in response to long stimulation pulses. It would appear that the contribution of such neurons is weighted more heavily in the LH reward substrate than in the PFC reward substrate.

253.5 AMPHETAMINE-INDUCED CHANGES IN SEROTONIN METABOLISM: OPPOSITE EFFECTS IN HIPPOCAMPUS AND STRIATUM. Craig D. Applegate* and Ronald Kuczenski. Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232.

Results of previous investigations have suggested a contribution of central serotonin (5HT) systems to the alterations which occur in brain and behavioral functioning following the administration of low doses of d-amphetamine (AMPH). In the present study we have examined changes in 5HT metabolism in response to a low dose of AMPH as an initial attempt to characterize patterns of AMPH-induced changes in this measure in discrete regions of the forebrain and mesencephalon.

Male Sprague-Dawley rats were administered either saline or 0.6 mg/kg AMPH subcutaneously and sacrificed by decapitation 15, 30 or 45 minutes later. Brains were rapidly removed and dissected over ice after the method of Segal and Kuczenski (Brain Res. 68, 1974). Levels of 5HT and its metabolite 5-hydroxyindole acetic acid (5HIAA) were assessed in brain tissue samples from prefrontal cortex, nucleus accumbens, striatum, hippocampus and substantia nigra via high pressure liquid chromatography (electrochemical detection) using a modification of the methods of Magnusson et al. (J. Chromatog. 221, 1980).

AMPH administration resulted in a significant decrease in 5HIAA levels in the hippocampus and a significant increase in striatal 5HIAA levels. The decreases in hippocampal 5HIAA were maximal at 15 minutes (87% control), were partially recovered by 45 minutes (92% control) and occurred in the absence of changes in 5HT levels. Striatal increases in 5HIAA levels were maximal at 30 minutes (116% control), were partially recovered at 45 minutes (113% control) and were accompanied by significant increases in 5HT levels. Maximal increases in striatal 5HT (133% control) occurred at 30 minutes following AMPH administration and were completely recovered (105% control) by 45 minutes. Levels of 5HIAA in the accumbens were decreased and increased at early and late time points, respectively ($p = .08$), while cortical 5HIAA tended to increase with maximal effects observed at 30 minutes. 5HT levels were unchanged in either of these regions, and levels of 5HT and 5HIAA were unchanged in the substantia nigra.

Sources of 5HT for the hippocampus and the striatum are primarily from the median and dorsal raphe, respectively. Opposite changes in 5HIAA levels produced by AMPH in these regions may reflect differences in the source of 5HT innervation to these structures. Relative contributions of the dorsal and median raphe innervation to accumbens and cortex may be reflected in the patterns of AMPH-induced change observed in these areas as well. (Supported by NIDA grant DA02676 and training grant MH15452).

253.6 REGIONAL DISTRIBUTION OF RADIOLABELED ZINC AND INULIN AFTER INTRACEREBROVENTRICULAR INJECTION IN THE RAT. E.G. Drust*, N.F. Harris, and I.L. Crawford, Depts. Pharmacology and Neurology, Univ. Texas Hlth. Sci. Ctr. at Dallas and VA Epilepsy Ctr., Dallas, TX 75216.

Zinc is distributed unevenly in the CNS and little information is available on processes regulating its concentration in the brain. The present study was designed to determine the distribution and accumulation of radiolabeled zinc (65Zn) in rat brain after intraventricular injection relative to the distribution of 14C-inulin, a marker of extracellular space. Rats received a lateral ventricular injection of an equimolar solution of zinc and inulin containing 500nCi 65Zn (277mCi/mmol) and 80nCi 14C-inulin (11mCi/mmol) and were sacrificed 1 or 4 days later. Brains were removed and dissected; eight brain regions were sampled. Tissues were placed in a gamma counter to measure 65Zn content and then solubilized for scintillation counting. Corrections were made for the Compton electron effect and for tissue quench. A portion of the data is summarized below.

REGION	DAY 1		DAY 4	
	[Zn]	Zn/In	[Zn]	Zn/In
Hippocampus	12.89±2.95	3.63	8.58±1.03	3.70
Cortex	8.06±2.69	2.09	4.25±1.28	2.41
Striatum	2.40±0.66	2.64	1.35±0.27	3.38
Cerebellum	0.58±0.18	2.32	0.13±0.04	----
Pons-Medulla	0.32±0.16	1.88	0.12±0.03	----

[Zn] = nmol/g wet weight ± SEM, n=5

Zn/In = pmol Zn / pmol Inulin accumulated

Radiolabeled zinc distributed in the brain in a pattern similar to that of endogenous zinc. The hippocampus, which contains the highest concentration of endogenous zinc in the regions assayed, accumulated the greatest concentration of 65Zn. Ratios of zinc/inulin accumulated indicate that the distribution of centrally administered zinc is not simply attributed to diffusion from ventricular CSF into extracellular space. In addition, measurements at day 4 indicate that the half-life of zinc is not uniform but is dependent on the region examined. These results suggest that the uptake, storage or utilization of zinc in the brain is not identical in all brain areas. This finding may be indicative of different physiological functions of zinc in specific brain regions. Supported by a VA Merit Review Research Program and NIH NIGMS GM07062.

253.7 MONOAMINE METABOLISM IN MICE TREATED NEONATALLY WITH MONOSODIUM GLUTAMATE (MSG). R. Dawson and Z. Annau. Div. of Toxicology, The Johns Hopkins Univ., Baltimore, MD 21205.

Neonatal administration of MSG to rodents results in destruction of the arcuate nucleus of the hypothalamus, obesity and endocrine dysfunction. The morphological and behavioral consequences of neonatal MSG administration have been extensively studied in the mouse. Few studies have utilized the mouse in assessing the neurochemical effects of MSG-induced arcuate nucleus damage. The present studies were undertaken to determine the effects of MSG treatment on monamine metabolism in the mouse.

Male and female CF-1 mice were treated on postnatal day 4 with a single subcutaneous injection of MSG (4mg/g) or saline. Monoamine metabolism was assessed in adults by measuring monoamine and metabolite levels by high performance liquid chromatography coupled with electrochemical detection under normal conditions and after challenges with α-methyl-p-tyrosine(MPT,400mg/kg), pargyline(100mg/kg) and reserpine(2mg/kg).

MSG-treated mice were found to have significantly lower levels of hypothalamic dopamine (DA), however 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) levels were normal,suggesting increased DA catabolism. MPT administration resulted in a slightly accelerated decline in hypothalamic DA levels when compared to controls. Pargyline-induced DA accumulation and HVA decline were also increased in MSG-treated mice. Control mice exhibited a 23% decline in DA levels 1 hour after reserpine treatment while MSG-treated mice were unaffected. MSG treatment did not alter hypothalamic norepinephrine (NE) or serotonin metabolism. Brainstem NE decline after MPT administration was significantly attenuated in MSG-treated mice. Acute administration of MSG to adult mice resulted in significant increases in hypothalamic levels of DA, DOPAC and 3-methoxy-4-hydroxyphenylglycol 1 hour after injection.

Neonatal administration of MSG results in depletion of hypothalamic DA and increased DA metabolism which may represent a form of neurochemical compensation. Acute administration of MSG to adults does not result in permanent alterations in DA metabolism but does exhibit a powerful pharmacologic action. The alterations in brainstem NE after neonatal MSG treatment represent a potentially important neurochemical effect of MSG.

253.8 EFFECT OF BENZTROPINE ON DOPAMINE(DA) UPTAKE IN A9,A10 AND DA POOR REGIONS M. G. Hadfield and E. A. Nugent*. Neurochemistry Research Laboratory, Section on Neuropathology, Department of Pathology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA. 23298

Recently, we reported that cocaine and amphetamine inhibit the synaptosomal uptake of DA less well in A10 regions (prefrontal cortex and olfactory bulbs) than in A9 regions (caudate-putamen). This could be due to (i) differences in A9 and A10 uptake mechanisms and/or (ii) non-specific effects such as uptake into non-DA elements. Specific uptake differences in central DA systems would be important because it might then be possible to selectively manipulate them pharmacologically. If similar differences are present in humans, the potential would exist to fine-tune neuroleptic and anti-parkinson medication to produce maximal clinical effects while reducing unwanted side-effects. A difference in uptake would fit well with the findings of others that central DA systems differ in other respects, such as the number, affinity and distribution of receptors and autoreceptors.

In the present study, we used benztropine because it is a more selective blocker of DA uptake than cocaine or amphetamine. DMI was simultaneously added to prevent DA uptake into NE terminals. The brains of adult male ICR mice were rapidly dissected over ice to obtain the pre-frontal cortex, olfactory bulbs, caudate-putamens, occipital cortex and cerebellum. Synaptosomes were prepared and incubated with tritiated DA (0.2 uM), DMI 10^{-8}M and varying concentrations of benztropine (10^{-7}M--10^{-2}M) were carried out according to the methods of Hendley et al.

At a concentration of 10^{-4}M benztropine produced virtually complete inhibition of DA uptake in the caudate-putamen. In all other tissues, uptake inhibition was far from complete. In the A10 regions (prefrontal cortex and olfactory bulbs) it was respectively 58-63% and in dopamine poor regions (occipital cortex and cerebellum) it was respectively 40% and 24%. These regions could only be pushed to a maximum uptake inhibition for DA at 10^{-3}M, respectively 76%, 71%, 71% and 53%. At 10^{-2}M there was a paradoxical rebound, or lessening of uptake inhibition to 70%, 51%, 64% and 41% respectively. The IC_{50} values are 2.5×10^{-6} for striatum, 2.5×10^{-5} for prefrontal cortex, 2×10^{-5} for olfactory bulbs, 2.5×10^{-4} for occipital cortex and 7.5×10^{-4} for cerebellum.

The results demonstrate differences in DA uptake inhibition for benztropine in various brain regions. Some of this may be due to specific differences because the IC_{50} values are roughly an order of magnitude apart for A9 DA vs A10 DA vs DA poor terminal regions. Yet it is obvious that some non-specific DA uptake is taking place except in the striatum. This is best demonstrated in the DA poor regions.

253.PO MULTIPLE BINDING SITES OF TRICYCLIC ANTIDEPRESSANTS: REGIONAL DISTRIBUTION IN RAT BRAIN. Anat Biegon, Hoffmann-La Roche Inc. Nutley NJ 07110.

The binding of ^{3}H-amitriptyline (Ami) and ^{3}H-Nortriptyline (Nor) was characterized on brain sections and the regional distribution of the binding sites analyzed by quantitative autoradiography using tritium sensitive (^{3}H-Ultrofilm LKB) sheet film and computerized densitometry. The binding of both drugs shows saturation at 0°C in the nM range. Atropine and mepyramine, a cholinergic muscarinic and a histaminergic agent, respectively, displace ami binding in an additive manner. Together, they account for 50% of the displaceable binding of 4 nM ami. The distribution of ami binding in the brain does not resemble that of any particular drug or transmitter system. However, in the presence of 200 nM atropine + 200 nM mepyramine, the distribution of the residual sites is similiar to that of 5HT and imipramine binding, being highest in the raphe superficial layers of the superior colliculus, interpeduncular nucleus, substantia nigra and mamillary nucleus.

Nor binding is highest in those brain regions enriched in norepinephrine and in DMI binding sites - namely the locus coeruleus, anteroventral thalamus, ventral bed nucleus of the stria terminalis. However, there are moderately high concentrations in the caudate and substantia nigra, which contain neither NE nor DMI binding. Nor labeling of these areas is reduced following a lesion of presynaptic serotonergic terminals with 5,7 dihydroxytryptamine. Displacement studies show both drugs possess a low affinity binding component, which is displaceable by nor, ami and other tricyclics only in μM concentration. In conclusion, amitriptyline and nortriptyline bind with high affinity to a number of different presynaptic and postsynaptic sites, and they share a low affinity binding component similiar to the one described for DMI (Biegon and Samuel, Biochem.Pharmacol. 28:3361, 1979) and imipramine (O'Brien et al., Soc. Neurosci. Abst. 4:430, 1978).

PRESYNAPTIC MECHANISMS I

254.1 PROTEIN I PHOSPHORYLATION IN PC12 CELLS. W.P. Melega* and B.D. Howard. Dept. of Biological Chemistry, UCLA Med. School, Los Angeles, CA 90024.

PC12 is a clonal line of rat pheochromocytoma cells that store and secrete dopamine and acetylcholine by processes that appear to be normal with one interesting exception. Whereas the evoked secretion of many compounds from a variety of secretory cells and nerve terminals requires ATP, the secretion of dopamine and acetylcholine from PC12 cells is not dependent on ATP (Biochemistry 21; 4795, 1982). One postulated role for ATP in secretion is as a substrate for protein kinase; certain proteins are known to be phosphorylated in secretory systems that have been stimulated to secrete. Perhaps the best characterized of these proteins are M_r 86,000 and M_r 80,000 proteins called proteins Ia and Ib, respectively, and found in nerve terminals of mammalian brain and peripheral nervous systems. We have studied protein phosphorylation in PC12. Stimulation of PC12 cells with 56 mM K^+ or 1 mM carbachol (conditions that evoke transmitter release from PC12) induces the detectable phosphorylation of only one protein, a M_r 60,000 protein that likely is tyrosine hydroxylase. PC12 cells contain proteins equivelent to mammalian brain proteins Ia and Ib; antibody raised against bovine brain proteins Ia and Ib cross reacts with PC12 M_r 86,000 and M_r 80,000 proteins as determined by immunoblotting. Like mammalian brain, PC12 cells contain more protein Ib than Ia. However, unlike the situation in mammalian brain, the PC12 protein Ib did not exhibit detectable phosphorylation. Cells were grown for 21 h in medium containing $^{32}P_i$, and a cell lysate was immunoprecipitated with antiserum against bovine protein I and Pansorbin. The immunoprecipitate contained both proteins Ia and Ib, but only protein Ia was phosphorylated. An interesting possibility is that the lack of detectable phosphorylation of protein Ib is related to the fact that the release of dopamine and acetylcholine from PC12 cells is independent of ATP stores. It may be that some modification of protein Ib in PC12 not only prevents phosphorylation of the protein, but allows protein Ib to perform whatever function it might have in transmitter release, even in the absence of phosphorylation.

254.2 EXCITABILITY CHANGES IN DOPAMINE-CONTAINING TERMINALS IN THE STRIATUM INDUCED BY CORTICAL STIMULATION. L. Chavez* and M. Garcia-Munoz. Dept. Neurosciences, Research Center in Cellular Physiology, U.N.A.M., Mexico.

Since glutamate is the neurotransmitter contained in cortical afferents to the striatum, and it has been shown that it induces dopamine release, both in vivo and in vitro, we decided to study changes in excitability, by electrically stimulating the dopamine-containing terminals within the striatum. The amount of current necessary to produce an antidromic action pot ential was recorded. As a depolarizing agent, glutamate 200 ng/ 0.2 ul, was injected into the frontal cortex. After cortical excitation, a mean of 21 ± 2 % increase in threshold was observed. In the cases where it was possible to follow the cell for a long time, it was observed that the change initiated its return to basal levels at 25 min post-injection. It is tempting to postulate that the dopamine release observed after glutamic acid, in other experiments, is accompanied by a decrease in terminal excitability.

254.3 **DUAL EFFECT OF LEAD ON TRANSMITTER RELEASE AT THE FROG NEUROMUSCULAR JUNCTION.** L. Kolton*, M.E. Selzer, and Y. Yaari* (SPON: M.S. Kreider). Department of Physiology, Hebrew University-Hadassah Medical School, P.O. Box 1172, Jerusalem, Israel 91010 and the Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Lead causes a decrease in EPP at low quantal content without affecting the quantal voltage, suggesting a presynaptic effect of lead (Manalis and Cooper, Nature 243:354, 1973). Using intracellular recordings, we have noticed that the reduction in endplate potential (EPP) at low lead concentrations was time dependent and that the depression was followed by an increase in EPP amplitude. We have examined this dual effect in more detail by sampling the endplate currents (EPC) of multiple endplates simultaneously with a surface-electrode at normal calcium concentrations (1.8 mM), and curare to eliminate muscle contractions. Curves of extracellular EPP amplitude versus log (Pb) were linear from 0.25-2 uM, but deviated from linearity in some experiments at 3-5 uM. This deviation was found to be due to the above mentioned time and concentration dependent increase in transmitter release.

When lead was removed by a specific lead chelator (2,3-mesodimercaptosuccinic acid) following an observed non-linear behavior, the EPP was increased above control. Moreover, the EPP showed superimposed population spikes, indicating that some muscle fibers had reached action potential threshold.

We conclude that lead has a dual effect on presynaptic transmitter release: an initial rapidly reversed reduction, and a delayed and longer lasting increase in transmitter release. Since these two effects can occur simultaneously, they probably occur at different presynaptic sites.

USPHS #F06 TW00697, the U.S.-Israel BSF and the Meller Endowment Fund.

254.4 FURTHER EVIDENCE FOR SEPARATE REGULATION OF MOTOR NERVE TERMINAL SIZE AND QUANTAL TRANSMITTER RELEASE AT MAMMALIAN NEUROMUSCULAR JUNCTIONS. D.S. Zahm* and Yong I. Kim. Department of Neurology and Clinical Neuroscience Research Center, University of Virginia School of Medicine, Charlottesville, VA 22908.

Numerous anatomical and physiological investigations have demonstrated a positive correlation between the diameter of a muscle fiber (MFD) and the quantum content (m) of the evoked transmitter release at low stimulation frequencies. Motor nerve terminal (MNT) size, expressed as the summed lengths of the branches of the terminal arborization, has also been thought to reflect m and therefore to be positively related to MFD. Grinnell and Herrera (J. Physiol. 307:301-317, 1980), however, demonstrated that two frog muscles with equal MNT sizes differed significantly in their respective m values, indicating that transmitter release/unit length of MNT is not necessarily uniform across different frog muscles. Differences in the ultrastructure and sprouting capacity of mammalian MNTs in muscles with different firing patterns or contraction properties have also been shown, but as yet MNT structure in mammals has not been investigated with respect to m.

We investigated MNT size, MFD, and m in several rat muscles. MNT sizes were measured on light micrographs (1000X) of zinc iodide- osmium tetroxide (ZIO) impregnated MNTs using the Zeiss Videoplan Image Analysis (ZVIA) measuring program. Diameters were estimated from micrographs of unsectioned, ZIO impregnated material and calculated as a function of the area of transversely sectioned, aldehyde-fixed, plastic embedded muscles using ZVIA. Quantum content was estimated by either the direct method using buffer containing 10 mM Mg^{++} or by the indirect method in curarized muscle. Conventional intracellular recording methods and a microcomputer system programmed for on-line aquisition and analysis of the junctional biopotentials and m were used.

MFDs from the flexor digitorum longus (FDL), soleus (SOL), sternocostalis, and focally innervated fibers of the superior oblique extraocular muscle (EOM) formed an ascending continuum with a range of about 10 to 75 μm. Quantum content measured both ways formed parallel ascending series of values. Mean MNT size for each muscle, however, appeared to be unrelated to any trend in m or MFD values. There was no significant difference in the mean size of EOM and FDL MNTs although m in FDL exceeded that in EOM by a factor of 2-2.5. SOL MNTs were the largest and most elaborate, but SOL m values did not exceed those of FDL. Within muscles, however, estimated MFD and MNT sizes were poorly but positively correlated. Several presynaptic factors, i.e., stimulation pattern and rate, transmitter storage and mobilization requirements, motor neuron size, or motor unit size may contribute to the regulation of MNT size. MFD and m alternatively may be co-regulated, possibly by predominantly postsynaptic factors.

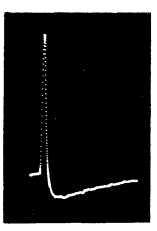
254.5 PRESYNAPTIC CURRENTS IN NEWLY FORMED NEUROMUSCULAR JUNCTIONS IN THE MOUSE. D. Angaut-Petit* and A. Mallart. Lab. Neurobiologie Cellulaire, C.N.R.S., 91190 - Gif s/yvette, France,

Uneven ionic channel distribution has been shown to exist along branches of adult mouse motor endings : Na channels are exclusively located close to the myelin while K channels are located only at the remainder part of the terminal. Thus, presynaptic currents recorded at the proximal parts show two negative components which correspond respectively to inward active Na current and inward capacity current caused by ionic outward K current at the remainder of the terminal. Distal signals consist in two positive deflections which are mirror images of those from proximal parts (Brigant and Mallart, J. Physiol., 333 : 619, 1982). We performed an in vitro electrophysiological study of channel distribution on regenerating motor endings in triangularis sterni muscle 13-14 days after nerve crush. To this purpose presynaptic membrane currents elicited by nerve stimulation were recorded by focal electrodes positioned under precise visual control (X 500).Signals recorded in our experimental conditions differed from those of adult endplates by two features. First, initial negative and distal positive late waveform components which signal K current were missing in many endplates. Absence of K dependent waveform components does not necessarily indicate absence of K current but, rather an even K current density in both proximal and distal parts. This hypothesis was tested by TEA+3,4 DAP ionophoresis close to recording electrode which revealed inward capacity current promoted by K outflux at adjacent regions of the terminal. Second, the sharp negative deflexion which has been shown to correspond to active inward Na current by its sensitivity to ionophoretic TTX application, could be recorded from a considerably enlarged length of the terminals.

In conclusion, regenerating motor endings do not show the sharp Na-K channel separation typical of mature endings but rather channel overlap as described in regenerating myelinated axons (Ritchie, Proc. R. Soc. Lond. B, 215 : 273, 1982 ; Kocsis et al., Proc. R. Soc. Lond. B, 217 : 77, 1982). Supported by the INSERM grant n° 816003.

254.6 INTRACELLULAR RECORDING OF ACTION POTENTIALS FROM VERTEBRATE NERVE TERMINALS USING POTENTIOMETRIC OPTICAL PROBES. A.L. Obaid*, B.M. Salzberg, H. Gainer, and D.M. Senseman*. Department of Physiology and Pharmacology, School of Dental Med. and Institute of Neurological Sciences, University of Pennsylvania Philadelphia, PA. 19104 and Lab. of Neurochemistry and Neuroimmunology, National Institutes of Health, Bethesda, MD. 20205.

Progress in achieving a better understanding of the physiology of synaptic transmission in vertebrates has been slowed by our inability to measure rapid changes in the membrane potential of nerve terminals. The giant synapse of the squid has provided a successful invertebrate model for the study of the release of transmitter substances because both the postsynaptic and the presynaptic membranes are accessible for electrophysiological study and intervention, including voltage clamp. Much of our knowledge of vertebrate synaptic physiology derives from the study of the frog neuromuscular junction but here "the small size of the nerve terminal prevents a direct measurement of the presynaptic potential change" (Katz, 1969). Optical methods that employ potential sensitive molecular probes are shown here to monitor rapid changes in membrane potential from a population of nerve terminals in the posterior pituitary (neurohypophysis) of amphibia, and the effects on the shape of the nerve terminal spike of extracellular calcium and other agents known to affect transmitter release may be studied. Calcium antagonists such as cadmium and nickel are shown to block a component of the action potential that probably reflects a calcium mediated potassium conductance and Tetrodotoxin blocks an inward sodium current, revealing a small calcium component to the action potential upstroke. The ability to record membrane potential changes from secretory terminals should be a significant aid in the study of excitation-secretion coupling in vertebrates.



50 mSec

Optical Recording of the Action Potential in Nerve Terminals of the Amphibian (Xenopus) Neurohypophysis. Single sweep.

Supported by U.S.P.H.S. grant NS 16824.

254.7 CHANGES IN THE DURATION OF SYNAPTIC CURRENT DURING DEPRESSION, FREQUENCY FACILITATION AND PTP AT SYNAPSES RC1-R15 OF APLYSIA CALIFORNICA. G. Grenon* and J.P. Tremblay. Dept. Anatomy, Laval Univ., Québec, Canada, G1K 7P4.

Cell R15 of Aplysia californica was voltage clamped at -100mV. Synapse RC1-R15, located between a fiber of the right connective and cell R15, was stimulated repetitively 100 times at 1.5 Hz and 40 times at 0.033Hz. This stimulation produces a characteristic sequence of changes in the amplitude of the synaptic current of successive responses due to synaptic depression, frequency facilitation and posttetanic potentiation (PTP). During the stimulation at 1.5Hz the latency of the synaptic current increases significantly but returns rapidly to its initial value when the rate of stimulation is reduced to 0.033Hz. This increased latency may be due to a reduced speed of propagation of the presynaptic action potential. The duration of the growth phase (beginning of synaptic current to its maximum) and of the decay phase (maximum synaptic current (MSC) to 50% of it) were also measured. There is a significant correlation between the depression ratio (i.e. MSC of 2nd response at 1.5HZ, over that of the 1st response) and the ratio of the growth phase duration of these responses for 40 trains of stimulations obtained in 11 preparations. However there is no significant correlation between the depression ratio and the ratio of the decay phase duration. The growth phase and the decay phase were significantly increased during facilitation and PTP by 1.0 to 3.0ms. A significant coefficient of correlation was also obtained between the facilitation ratio (i.e. MSC of the hundredth response at 1.5Hz over that of the 1st response at 1.5Hz) and the ratio of the growth phase duration of these responses. The facilitation ratio was also significantly correlated with the ratio of the decay phase duration. Similarly the PTP ratio (i.e. MSC during the 1st response after the train at 1.5Hz over that of the 1st response at 1.5Hz) was also significantly correlated with the ratio of the growth phase duration and with the ratio of the decay phase duration of these responses. These results indicate that the increased duration of the growth phase and of the decay phase of the synaptic current contribute to the facilitation and the PTP phenomena.

The increased duration of the growth phase and of the decay phase observed in these experiments is probably due to an increased duration of the neurotransmitter release. This may be due to a widening of the presynaptic spike such that each presynaptic bouton liberates the neurotransmitter during a longer period. These increased durations may also be due to an invasion of more branches of the terminal arborization such that although the release period is the same in each bouton, the total duration of the release period is increased due to the non synchronous invasion of all the participating boutons.

254.8 MINIMAL OR NO POSTSYNAPTIC CONDUCTANCE CHANGES IN MOTONEURONS DURING PRESYNAPTIC INHIBITION IN CHLORALOSE ANESTHETIZED CATS, D.A. McCrea*, P.L. Carlen. Playfair Neuroscience Unit, Toronto Western Hospital, and Addiction Research Foundation Clinical Institute, Depts. of Physiology and Medicine, University of Toronto, Ontario, Canada.

Eide et al, (Structure and Function of Inhibitory Neuronal Mechanisms, Pergamon Press, pp. 215-219, 1968) reported that conditioning stimuli which were effective in reducing the amplitude of the monosynaptic IaEPSP produced by stimulation of low threshold muscle afferents, did not produce a change in the membrane conductance of the motoneuron (MN) and thus provided evidence that the inhibition of the IaEPSP was presynaptic (PSI). Using the more sensitive techniques of signal averaging and the measurement of focal conductance changes by estimating the changes in membrane time constants with short current pulses, Carlen et al. (J. Physiol. 298: 539, 1980) were able to detect changes in MN conductance as well as small long-lasting IPSPs during presynaptic inhibition. These authors sugested that these changes were not insignificant and could be responsible for the small reduction in the amplitude of the IaEPSP produced by the PSI seen using barbiturate-anesthetized cats.

We have re-examined this question of postsynaptic phenomena accompanying and perhaps being responsible for PSI in cats anesthetized with alpha-chloralose. In this preparation we have seen as much as a 60% reduction in the size of the IaEPSP in medial and lateral gastrocnemious and hamstring MNs produced by preceding the test IaEPSP with a train of 5 stimuli (300 hz) to the nerve from the hamstring muscles at a condition-test interval of 50 to 70 ms. We have used both short (50-80 nA, .2ms) and long (2 nA, 30ms) hyperpolarizing pulses to measure the conductance changes produced by the conditioning stimuli and have found very minor or no conductance increase at a condition-test interval that produced a large reduction in the IaEPSP amplitude. In addition, we have examples in which there is no detectable IPSP (i.e. < .2mV) with a large reduction in the size of the IaEPSP. We conclude that in chloralose-anesthetized cats, while there may be small postsynaptic events occuring during PSI, they are of insufficient magnitude to account for such large reductions in the IaEPSP amplitude, as compared to barbiturate-anesthetized cats. In this case, the most likely location for the phenomenon causing PSI is indeed presynaptic. (Supported by MRC 6019).

254.9 STUDIES OF MINIATURE ENDPLATE CURRENTS SHOW THAT THE QUANTUM OF RELEASE IS COMPOSED OF SUBUNITS. C. Erxleben*, G. Carlson*, and M.E. Kriebel* (Spon: R. King) Dept. of Physiol., Upstate Medical Center, SUNY, Syracuse, NY 13210.

Miniature endplate potential (MEPP) amplitude histograms show integral peaks that are multiples of the first peak (s-MEPP class) and these peaks remain stationary over time (Kriebel, et al., 1976, J. Physiol. 262:553). Unitary evoked endplate potential (EPP) histograms match those of MEPPs showing that unitary EPPs and MEPPs have the same subunit (Kriebel, et al., 1982, 322:211). However, Magleby and Miller (1981, J. Physiol. 311:267) were not able to find stationary and integral peaks of miniature end plate potential amplitude distributions. We voltage clamped (-140 mV) mouse diaphragm junction with 2 electrodes. Flectrode (5 megohm, 8 M CsCl) sealing and low holding currents were achieved with a 25 mM Ca^{++} saline at 20°C or 30°C. With these conditions MEPc frequencies (20/sec) were appropriate to collect 8000 MEPcs from a single junction. The mean MEPc (3.5 nA) to noise (35 pA rms) ratio was 1%. Signals were stored on magnetic tape and MEPc amplitude, time-to-peak, rise time, net charge, falling phase and baseline noise were determined for each MEPc with a computer program. The falling phases of s-MEPcs and bell-MEPcs were single exponential curves. MEPc amplitude histograms showed integral peaks and peak stationarity. We found that a bin size 1/50th that of the mode MEPc and 1.5 x base line noise produced smooth peaks separated by 5 or more bins when the sample size was large enough to yield 50 MEPcs in each bin. Thus, random variations due to small sample size neither obscured integral peaks nor produced spurious peaks; and, 1200 MEPcs were adequate to define the central 3 or 4 peaks. The peak intervals with identical conditions were constant within and between preparations although the number of peaks varied (range of modes 8 to 12, adult). The post-synaptic effects of either different temperatures or eserine were found to change peak intervals but not the number of peaks. Eserine increased the peaks of the MEPP amplitudes by about 80%. Decreasing the temperature from 30°C to 20°C decreased the MEPcs by 30%. The integral peaks of MEPc amplitude histograms demonstrate a physiological subunit of the quantum of transmitter release. Because of the small variance of each peak and large vesicle volume variance (see Hanna and Kriebel, this volume), it appears improbable that the subunit represents the release of free ACh from a vesicle. The small variance of each peak, which is the result of pre- and post-synaptic variance, could be explainable with macromolecules that bind a constant number of ACh molecules so that the subunits correspond to the number of macromolecules involved in the release process.

254.10 DEPRESSION AND RECOVERY OF NEUROMUSCULAR TRANSMISSION DURING PROLONGED TETANIC STIMULATION. Toshio Narahashi and Mladen I. Glavinovic. Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611, and Depts. of Anaesthesia and Physiol., McGill Univ., Montréal, Québec, H3G 1Y6, Canada.

It has generally been believed that synaptic depression as a result of prolonged presynaptic tetanic stimulation at high levels of transmitter release is due to insufficient replenishment of the store of transmitter immediately available for release (n). This was supported by statistical analysis, which showed that depression was associated with decrease in n and not in p (probability of release) which remained largely unchanged. One can directly estimate the changes in the depletion and replenishment of the immediately available store from the changes in the synaptic output.

End-plate potentials (EPPs) were recorded from the unparalyzed "cut" rat phrenic-diaphragm preparation. Short tetanic stimulation (50 Hz, 500 msec) were applied to the nerve at a frequency of 1 Hz. During the initial tetanic stimulation the amplitude of EPPs first increased and then decreased reaching an apparent steady-state level. Recovery of EPPs occurred during the following 500 msec rest period as indicated by the amplitude of the first EPP in the subsequent, second train of tetanic stimulations. During the second train, the EPP amplitude again decreased reaching a new apparent plateau level. This pattern of change in EPP amplitude remained throughout a series of tetanic stimulations. The amplitudes of both the first and the last (25th) EPPs during a series of short tetanic stimulations decreased as the stimulation proceeded. Surprisingly, the amplitude of the last EPPs decreased faster than that of the first. During a series of tetanic stimulations, there was an increase in the relative depression which is defined by the ratio of the difference between the first and last EPPs in a train to the first EPP. An increase was also observed for the relative recovery which is defined by the ratio of the difference between the first EPP in a train and the last EPP in a preceding train to the first EPP of the preceding train. A model suggested before by Glavinovic (J. Physiol. 290, 481, 1979) can be used to explain the observed phenomena. According to this model, two processes occur simultaneously during the tetanic stimulation. In addition to the depletion of the immediately available store as well as the store behind it, there is an increase in the capacity of the immediately available store to contain transmitter (presumably caused by entry of some substance such as calcium). As a result, the immediately available store is rapidly replenished during recovery, although the store behind it is still largely depleted. Supported by MRC and MDA of Canada to MIG and by NIH, NS14145, to TN.

254.11 SYNAPSIN I (PROTEIN I) BINDS SPECIFICALLY AND WITH HIGH AFFINITY TO HIGHLY PURIFIED SYNAPTIC VESICLES FROM RAT BRAIN.
W. Schiebler*, J. Rothlein, R. Jahn*, J. P. Doucet* and P. Greengard (SPON: C. Romano). Dept. Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510.

Synapsin I (Protein I) is a neuron-specific phosphoprotein present in most, and possibly in all, synaptic nerve endings, where it appears to be associated primarily with synaptic vesicles (De Camilli et al., J. Cell. Biol., 96, 1337 & 1355, 1983). It is a prominent substrate for cAMP-dependent and Ca^{2+}/calmodulin-dependent protein kinases (Huttner, DeGennaro and Greengard, J. Biol. Chem., 256, 1482, 1981). We have now investigated the interaction between homogeneous Synapsin I and purified synaptic vesicles in vitro.

Synapsin I was purified to homogeneity from rat brain synaptosomes by a novel procedure, including a salt-detergent extraction with 150 mM NaCl/1% CHAPS and purification through CM-cellulose- and hydroxylapatite-columns (Doucet, Schiebler and Greengard, manuscript in preparation). Synaptic vesicles were purified from rat brain synaptosomes using techniques of sucrose density gradient centrifugation and of chromatography on controlled pore glass beads (Huttner, Schiebler, Greengard and De Camilli, J. Cell Biol., 96, 1374, 1983). Binding experiments were performed by ultracentrifugation and/or centrifugation/gel filtration through Sephacryl S-300 columns. The amount of bound Synapsin I was determined by immunological methods.

For these binding studies, synaptic vesicles were depleted of endogenous Synapsin I by diluting and washing with 200 mM NaCl or 200 mM KCl. Binding of Synapsin I to Synapsin I-depleted synaptic vesicles demonstrated time-dependent, saturable binding, which was not seen in experiments using Synapsin I-containing vesicles or control membranes such as membranes derived from human red blood cells and rat brain nerve endings. Binding was found to be dependent on ionic strength. In 40 mM salt, the K_d for binding was 3 (±2) nM (measured by centrifugation/gel filtration and/or ultracentrifugation). At physiological ionic strength (equivalent to 150 mM NaCl), the K_d increased approximately threefold (measured by ultracentrifugation). The amount of Synapsin I bound to synaptic vesicles at saturating concentrations was comparable to the amount of endogenous Synapsin I found in non-depleted vesicles. We are currently investigating the regulation of the binding of Synapsin I to synaptic vesicles. These studies include examining the effect, on binding, of phosphorylation of Synapsin I at distinct sites by cAMP-dependent and Ca^{2+}/calmodulin-dependent protein kinases.

254.12 A PRESYNAPTICALLY TARGETED NEUROTOXIN THAT BLOCKS VARIOUS CHEMICAL SYNAPSES IN FROG.
Doju Yoshikami, Lynne M. Kerr and Keith S. Elmslie* (SPON: M. McPheeters). Dept. of Biology, University of Utah, Salt Lake City, Utah 84112.

We reported at these meetings last year that a small, basic neurotoxic peptide from the marine snail, Conus geographus, irreversibly inhibits synaptic transmission in skeletal muscles of frog by blocking transmitter release. This peptide, called ωCgTX, also irreversibly blocks the Ca^{++} component of the action potential in dorsal root ganglion (DRG) neurons from embryonic chick. We report here that ωCgTX irreversibly blocks synaptic transmission in the sympathetic ganglion and spinal cord of frog.

Sympathetic ganglion: Extracellular recordings from isolated preparations reveal that within minutes of the application of μM concentrations of ωCgTX, ganglionic transmission is totally and irreversibly blocked. Intracellular recording from principal neurons within the ganglion show that upon ωCgTX treatment, antidromic responses remain unaltered at a time when orthodromic responses are totally eliminated. This shows that ωCgTX blocks synaptic transmission without affecting the propagation of action potentials in the ganglion. Furthermore, during the course of toxin action when the block is incomplete, the quantal content of transmitter release is reduced. Thus, ωCgTX produces a state resembling that normally achieved by lowering $[Ca^{++}]_o$. In this regard, ωCgTX blocks ganglionic and neuromuscular synapses similarly, and it does so apparently by preventing Ca^{++} entry into the nerve terminal during the presynaptic action potential.

Spinal cord: Extracellular potentials from the ventral root of an isolated, hemisected lumbar spinal cord were monitored. The response produced by stimulation of the dorsal root consists of multiple components and reflects contributions from presynaptic action potentials as well as synaptic potentials. Addition of ωCgTX to the bathing medium exclusively blocked all synaptic components. In this respect, the effect of ωCgTX is the same as that produced by exposing the preparation to low $[Ca^{++}]$-high $[Mg^{++}]$. Unlike low $[Ca^{++}]$-high $[Mg^{++}]$ however, the synaptic block produced by ωCgTX was not reversible. It is also notable that the response to bath-applied quisqualate, an excitatory amino acid agonist, was not affected by ωCgTX treatment. This result is consistent with the hypothesis that ωCgTX does not act post-synaptically at CNS synapses, but rather its site of action is presynaptic -- just as it is for peripheral synapses.

We thank Dr. B. M. Olivera & W. R. Gray of our Department for supplying us with pure ωCgTX. This research was supported by PHS grants NS15543, NS00465 and an MDA Postdoctoral Fellowship to L. M. K.

254.13 SKATE ELECTRIC ORGANS GENERATE TWO CLASSES OF SPONTANEOUS MINIATURE ELECTROCYTE JUNCTION POTENTIALS.
C.E. Gross* and M.E. Kriebel* (Spon: J.B. Preston) Dept. Neurosurg. Un. Col., Denver, CO 80220 and Dept. Physiol., Upstate Medical Center, SUNY, Syracuse, NY 13210.

The frog neuromuscular junction generates two classes of miniature endplate potentials (MEPPs) and the ratio of small MEPPs (s-MEPPs) to bell-MEPPs (classical) can be changed with periods of elevated temperature, calcium or nerve stimulation (Kriebel and Gross, 1974, J. Gen. Physiol. 64:85, Kriebel, 1978, Brain Res. 148:381). Electric organs have been extensively used in acetylcholine and isolated synaptic vesicle studies; and, although spontaneous potentials have been reported they have not been studied in detail. Since electrocytes are embryologically like muscle and are richly innervated we report the amplitude distribution of spontaneous miniature electrocyte junction potentials (MEJPs). A two mm thick section of skate tail (R. binoculata) was pinned to a small bath containing elasmobranch saline (room temp). Microelectrodes (3 M KCl) were lowered perpendicularly to the surface of the electric organ and signals were recorded on magnetic tape and a chart recorder or from an oscilloscope with film. Because of the low electrocyte input resistance, MEJPs recorded from the interior were very small and usually skewed to the noise. However, with 50 K ohm microelectrodes (20 μV peak-to-peak noise) we found that MEJP amplitude histograms had a peak at 60 μV and showed a slight right-hand skew with MEJPs up to 1 mV. When recording microelectrodes were advanced against the innervated membrane from either outside or from inside the cell, we recorded identical focal potentials (only polarity reversed) with means of 3 to 5 mV. Most of these were generated at the electrode tip and their frequencies were increased with electrode pressure. Focally recorded MEJPs generated at the electrode tip are readily distinguishable from those generated at remote junctions which have slow time characteristics. At 10°C, mainly focal MEJPs with fast time characteristics were recorded. MEJP amplitude histograms of focally recorded MEJPs with fast rise times show two bell-shaped peaks. The small mode MEJP peak was about 1/20th the amplitude of the larger class of MEJPs (bell-MEJPs). These small mode MEJPs (s-MEJPs) were lost in the noise when the electrode tip was internal. The ratio of s-MEJPs to bell-MEJPs was variable since the bell-MEJP frequency was more dependent on the electrode pressure. Graphs of time-to-peak vs. amplitude show that both classes of MEJPs fall on the same line. We suggest that the s-MEJPs recorded from the electrocytes are similar to the s-MEPPs recorded from frog and mouse neuromuscular junctions.

254.14 Na-DEPENDENT NOREPINEPHRINE RELEASE FOLLOWS INHIBITION OF THE Na,K-ATPase IN SYMPATHETIC NEURONS.
Kathleen J. Sweadner. Neurosurgical Research, Mass. General Hospital, Boston MA 02114.

Ouabain, a specific inhibitor of the Na,K-ATPase, has been previously found to elicit release of transmitter by a Ca^{+2}-independent mechanism in a variety of systems. There are two different Na,K-ATPases in the nervous system which differ in their affinities for ouabain (Sweadner, 1979. J. Biol. Chem. 254:6060). Many, if not all, neurons of the CNS express a high affinity form, but sympathetic neurons express the same low affinity form that is found in the heart. Ouabain is best known for its ability to cause inotropy (increased contractility) in the heart. Other investigators have shown that the inotropic effect of ouabain occurs at concentrations well below those required to inhibit the Na,K-ATPase 50% in vitro; thus the increase in contractility precedes symptoms of toxicity. The mechanism may involve the indirect regulation of intracellular Ca^{+2} levels through an alteration of intracellular Na^+ levels. In search of an analogous effect on transmitter release, the ionic basis of the vesicular release of norepinephrine (NE) was studied in primary cultures of sympathetic neurons.

First, the Ca^{+2} and Na^+ dependence of classical stimulants was tested. When NE release was elicited by 54mM K^+ it was dependent on extracellular Ca^{+2}, and was not blocked by TTX or by reducing extracellular Na^+ to 14 mM (substituting choline). When NE release was elicited with the Ca^{+2} ionophore A23187 it was not dependent on extracellular Ca^{+2}, presumably because it penetrates the cell and releases Ca^{+2} from intracellular stores. Release elicited by A23187 was enhanced in 14mM Na^+, presumably because 14mM Na^+ reduces efflux of Ca^{+2} by Na:Ca exchange. Release was also elicited by veratridine and blocked by TTX, and this was independent of extracellular Ca^{+2}.

The Na,K-ATPase was inhibited by ouabain or by removing extracellular K^+ (substituting Na^+). In both cases, NE release was independent of Ca^{+2}. TTX, however, was able to delay release by hours, and 14mM Na^+ blocked the release entirely. The implication is that the effect of inhibition of the Na,K-ATPase on release is secondary to the entry of Na^+.

The concentration of ouabain and of extracellular K^+ required to elicit NE release was compared to the concentrations required to inhibit Na,K-ATPase activity. Little or no release was seen below a threshold of 70-80% inhibition. Release was followed by a marked morphological deterioration of axonal processes as well, indicating that it is part of a pathological process unlike inotropy. (Supported by NS 18233; Established Investigator of the American Heart Association)

254.15 THERMODYNAMIC ANALYSIS OF THE BINDING OF A CALCIUM ANTAGONIST TO SYNAPTOSOMAL MEMBRANES. G. A. Weiland and R. E. Oswald. Department of Pharmacology, Cornell University, Ithaca, NY 14853.

Radiolabeled calcium antagonists such as [^{3}H]nitrendipine have been utilized to identify putative calcium channels in membranes from a number of tissues. The binding of radiolabeled 1,4-dihydropyridines has yielded information regarding the interaction of calcium antagonists with their binding sites, including the dependence of binding on divalent cations. To study the mechanism of the binding reaction, the temperature dependence of the binding of [^{3}H]nitrendipine to brain membranes was examined and the thermodynamic properties characterized.

Synaptosomal membranes were prepared from rat brain using the Ficoll gradient flotation method. [^{3}H]Nitrendipine binding was determined in the presence of 1 mM calcium, using a rapid filtration assay. The radioligand bound specifically with high affinity (K_D = 0.61 nM) to a single class of sites on the membranes (B_{max} = 550 fmol/mg protein) and was displaced by nanomolar concentrations of unlabeled nitrendipine. Kinetic analysis of the association and dissociation rates was consistent with a simple bimolecular reaction (k_{-1}/k_1 = 0.88 nM).

The temperature dependence of the equilibrium constant and the association and dissociation rates for [^{3}H]nitrendipine binding were examined between 0°C and 25°C. Significant loss of binding was observed at 37°C, probably reflecting binding site lability at elevated temperatures. The binding was almost independent of temperature over this range, the equilibrium and kinetic constants increased only 2- to 3-fold with the increase in temperature. Thermodynamic analysis of binding at equilibrium demonstrated approximately equal changes in enthalpy and entropy ($\Delta H°$ = -5.0 kcal/mole, $\Delta S°$ = +24 e.u., $-T\Delta S°$ = -7.05 kcal/mole). Thus there is probably a significant contribution of hydrophobic bonding to the reaction, as has been observed for many binding reactions. The activation energies were 6.1 kcal/mole (ΔH^+ = 5.4 kcal/mole, ΔS^+ = -13 e.u.) for association and 10.6 kcal/mole (ΔH^+ = 10.0 kcal/mole, ΔS^+ = -39 e.u.) for dissociation. Thus the enthalpic and entropic contributions to the transition state barrier are approximately equal. These results will be discussed in terms of the molecular mechanism of nitrendipine binding to the putative calcium channel and of the effects of divalent cations on the calcium antagonist binding site.

Supported by a PMA Foundation Career Development Award and Starter Grant (G.A.W.) and NIH BRSG 08-S7RR05462D-20 (R.E.O.).

254.16 **TEMPORAL CHARACTERISTICS OF POTASSIUM-STIMULATED ACETYLCHOLINE RELEASE AND INACTIVATION OF CALCIUM INFLUX IN RAT BRAIN SYNAPTOSOMES.** M.E. O'Leary* and J.B. Suszkiw. Dept. of Physiology, Univ. of Cincinnati, Coll. of Medicine, Cincinnati, OH 45267.

The time-course of Ca^{2+}-dependent [^{3}H] ACh release and inactivation of ^{45}Ca entry were examined in rat brain synaptosomes depolarized by 45mM $[K^+]_o$. Under the conditions where the intrasynaptosomal stores of releasable [^{3}H]ACh were neither exhausted nor replenished in the course of stimulation, the K^+-evoked release consisted of a major (40% of the releasable [^{3}H]ACh pool), rapidly terminating phase ($t_{\frac{1}{2}}$=17.8 sec), and subsequent, slow efflux which could be detected only during a prolonged, maintained depolarization. The time-course of inactivation of K^+-stimulated ^{45}Ca entry suggests the presence of fast-inactivating ($t_{\frac{1}{2}}$<5s), slow-inactivating ($t_{\frac{1}{2}}\sim$40 sec) and a noninactivating, or very slowly inactivating ($t_{\frac{1}{2}}$>300sec), components.

The present data do not permit unequivocal determination which component of Ca^{2+} -entry in synaptosomes is instrumental in the phasic release of [^{3}H] ACh. Nevertheless, since inactivation of that Ca^{++}-entry pathway with which transmitter release is associated should precede inactivation of transmitter release, it is probable that only the "fast" inactivating pathway is involved in the fast phase of transmitter release. The noninactivating Ca^{++}-entry may account for the slow phase of transmitter release. These results indicate that under conditions of maintained depolarization of synaptosomes by high $[K^+]_o$ the time-course and the amount of transmitter released may be a function of the kinetics of inactivation of the voltage-dependent Ca-channels. (Supported by NIH grant NS17442)

254.17 LOCALIZATION OF CALCIUM ENTRY IN THE AXONAL TERMINALS OF MEDIAL PHOTORECEPTORS OF THE GIANT BARNACLE DETECTED WITH ARSENAZO III. N. Stockbridge* and W.N. Ross. Department of Physiology, New York Medical College, Valhalla, NY 10595.

Previous studies have shown that there is a calcium current in the terminal region of the decrementally conducting medial photoreceptor axons of the giant barnacle, Balanus nubilus. But these studies had poor spatial resolution (Ross and Stuart (1978) J. Physiol. 274: 173-191; Edgington and Stuart (1979) J. Physiol. 294: 433-445). We have used the calcium indicator dye Arsenazo III to examine the spatial distribution of calcium influx in the photoreceptor. A study of calcium influx and removal may aid understanding of how transmitter release at this tonically active synapse differs from the phasic release seen, e.g. at the squid giant synapse.

The ocellus containing the medial photoreceptors and the supraesophageal ganglion to which they project were mounted in a chamber on the stage of a Zeiss compound microscope. Arsenazo III (75 mM) was iontophoresed into the 20 um diameter region of a photoreceptor axon and allowed to diffuse several hundred microns to much finer presynaptic terminals in each hemiganglion. The preparation was imaged upon a 100 element photodiode grid permitting spectrophotometric measurements with 25 um resolution. Later filling of the cell with Lucifer Yellow permitted close correlation of the anatomy with the spectrophotometry.

In response to a depolarization produced by brief light flashes or electrically via the microelectrode, an absorbance increase in each terminal was observed at 660 nm, a smaller absorbance decrease was observed at 532 nm, and no signals were apparent at either 577 or 750 nm, all of which is consistent with an increase in ionized calcium interacting with the dye. Absorbance changes were detectable even when the dye concentration in the terminals was too low to see. These changes were larger when calcium action potentials were induced by bathing the preparation in saline containing 50 mM TEA and could easily be observed without averaging.

Detectable absorbance changes were confined to the region of the terminal arborization, typically to within the final 100 um. Even within that distance, most of the signal was confined to the last 50 um. Within 50 um of this focus, the signals fell at least 25-fold. This was true even though any concentration gradient in Arsenazo III should have been in the opposite direction.

Supported by USPHS grant NS 16295 to WNR, NRSA Fellowship NS07172 to NLS, and an Irma T. Hirschl Career Scientist Award to WNR.

254.18 INTRACELLULAR RECORDINGS FROM SYNAPTIC TERMINALS DURING FACILITATION OF TRANSMITTER RELEASE. George D. Bittner and Douglas A. Baxter. Department of Zoology, University of Texas at Austin, Austin, TX 78712.

Crustacean neuromuscular junctions exhibit an impressive range of use-dependent changes in synaptic efficacy. During a brief, high-frequency train of action potentials (APs), the amplitude of the excitatory junctional potentials (EJPs) can increase as much as 600-fold. This phenomenon, called short-term facilitation (STF), decays with time course of about 700 msec. STF can be shown to result from a presynaptic increase in the mean quantal content. However, little is known about possible electrophysiological changes in the nerve terminals that may accompany these phenomena. We have therefore made intracellular presynaptic recordings from the terminals of the opener-excitor motor neuron of crayfish during and after tetanic stimulation of the axon.

Crayfish (P. simulans) opener muscle fibers are innervated by a single excitor axon. By removing the closer muscle it is possible to expose the synaptic terminals of the opener-excitor as it branches over the ventral surface of the opener muscle. Primary, secondary, or tertiary nerve branches can be suspended above the muscle surface and penetrated with 30-60 MΩ, 3 M KCl microelectrodes. These recording sites are within 100-300 µm of nerve-muscle contacts and are electrotonically close (about 0.5λ) to presynaptic release sites.

APs at the terminal are 96 ± 4 mV (mean ± SD; N = 15), overshoot the -75 ± 7 mV rest potential, and are followed by a depolarizing afterpotential (DAP) of 10 ± 2 mV. The DAP has a duration of 30-50 msec and the AP afterpotential reverses polarity at about -65 mV. During stimulus trains of 10 impulses at 100 Hz, the peak voltage of the second and successive APs are between 2-10 mV larger than the first AP (mean = 5.7 ± 4 mV). In contrast, the total foot-to-peak amplitudes of the second through the tenth APs are reduced by an average of 6 mV. The duration of the APs increase by 18% (0.40 to 0.47 msec). Although the amplitude of the EJPs grow throughout the entire stimulus train, progressive increases in the peak voltages and the duration of APs never continue for more than 4 impulses. Hence it is unlikely that changes in AP amplitude are responsible for STF.

During prolonged stimulation (2-8 sec), the nerve terminals become hyperpolarized. This post-tetanic hyperpolarization decays with a time constant of 18 sec. Its amplitude (400 µV to 10 mV) is related to the frequency and duration of stimulation. This potential change may be responsible for a component of post-tetantic facilitation having a time constant of 10-40 seconds.

254.19 EFFECTS OF Pb^{2+} and Cd^{2+} ON TRANSMITTER RELEASE AND Ca^{2+} FLUXES IN NERVE TERMINALS. J.B. Suszkiw, G.P. Cooper and M. Murawsky*. Dept. of Physiol. and Environ. Health, Univ. of Cincinnati, Coll. of Med., Cincinnati, OH 45267.

These experiments examined the effects of Pb^{2+} and Cd^{2+} on: (a) transmitter release,in electrophysiological studies of the frog neuromuscular junction and (b) ^{45}Ca movements, in biochemical studies of rat brain synaptosomes. Pb^{2+} reduces (K_D, 1 µM) evoked transmitter release (endplate potential; EPP) and stimulates spontaneous quantal release (MEPP). Similar concentrations of Cd^{2+} block the EPP (K_D, 2.6 µM) but have no effect on MEPP frequency. The effects of Pb^{2+} and Cd^{2+} on the EPP are additive; however, Cd^{2+} actually reduces the Pb^{2+}-induced increase in MEPP frequency. Both Pb^{2+} (K_D, 2 µM) and Cd^{2+} (K_D, 3.7 µM) competitively block the K^+-depolarization-stimulated influx of ^{45}Ca in synaptosomes. In the subcellular fractions of nerve endings the following effects are observed: (a) Pb^{2+} and Cd^{2+} competitively inhibit the ATP-dependent accumulation of ^{45}Ca in cholinergic synaptic vesicles (K_D=50 µM, both metals); (b)in hypo-osmotically disrupted, NaN_3 poisoned synaptosomes, Cd^{2+} inhibits (K_D, 50 µM) the ATP-dependent uptake of ^{45}Ca but the principal effect of Pb^{2+} is the enhanced passive binding of ^{45}Ca. This effect is interpreted to result from the increased Ca-^{45}Ca exchange in subsynaptosomal Ca-sequestering structures (endoplasmic reticulum and/or mitochondria) and indicates a disruption of the Ca-permeability barrier in these subsynaptosomal structures by Pb^{2+}. However, this effect is prevented by Cd^{2+}. These results suggest that the differential effects of Pb^{2+} and Cd^{2+} on MEPP frequency can be explained by the Pb^{2+} (but not Cd^{2+})-induced leakiness of intracellular membranes to Ca^{2+}. The relatively high concentrations of the metal ions necessary to block ATP-dependent Ca-sequestration systems explain why Cd^{2+} is ineffective in increasing MEPP frequency. The block of Pb^{2+}-induced increase in MEPP frequency by Cd^{2+} may be explained by either its competition with Pb^{2+} at the external Ca-channel and/or by its negating effects on Pb^{2+}-induced Ca^{2+}-leakiness at the intracellular sites. Supported by NIH grants NS 17968 and ES 00159.

254.20 CALCULATION OF K-TRAPPING IN THE PSEUDOPODIAL INDENTATIONS FORMED BY ABUTTED NERVE TERMINALS. E.B. Lankford* and A.F. Boyne, Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD 21201.

It has been established that blind-ended pseudopodial indentations (PSI) form between abutted afferent nerve terminal swellings synapsing onto smooth dendrites in the globus pallidus and substantia nigra (Boyne and Tarrant J. Neurosci. 2, 463, 1982). The cavity is a diffusion trap because ions or solutes released non-synaptically during electrical activity of the parent nerve (e.g. action potential release of K^+) must diffuse along the extracellular space around the shaft of the pseudopodium before they reach the outlet to the three-dimensionally branching corridors of the general extracellular space. The highest concentrations of non-synaptically released substances will therefore develop at PSI tips. It has been suggested that overgrown PSI could predispose the substantia nigra to an irreversible loss of its inhibitory tone (Boyne and Phillips, Fed. Proc. 41, 2188, 1982). Hippocampal mossy fiber nerve terminals bear PSI; a possible role in the development of long term potentiation at this site is therefore also evident. We have sought a method of normalizing the varied geometries of individual PSI by calculating the expected K accumulations when firing at given frequencies. With reconstructions of real PSI, the complex geometries and complex boundary conditions make a closed form solution impossible. However, a finite difference equation is tractable using numerical techniques and computerized iterations. Three dimensional modelling of serial sections has been used to determine the overall shape of PSI. The K trapping can now be calculated by taking the dimensions from, and performing the calculations with, a number of segments which is equal to the number of cross sections prepared.

The results show that K accumulations which are known to modulate neuronal electrophysiological processes when applied in bulk solution (up to 10 mM; Sykova, J. exp. Biol. 95, 93: 1981) are attained at the tips of PSI of previously observed lengths (3 microns) over a firing frequency range of 50 to 200 Hz. This encourages the view that PSI, which are known to be plastic, could modulate nerve terminal electrophysiology by generating variable K 'hot spots' deep within the boutonal swellings. The conversion of complex geometry information to an applied K accumulation value shows that slight stimulation-induced changes in PSI size/shape would have more substantive effects on K trapping.

(Supported by U.S. Army Research and Development Command Contract No. DAMD 17-81-C-1279).

254.21 PRESYNAPTIC MODULATION OF ADRENERGIC NEUROTRANSMISSION IN THE RABBIT IRIS-CILIARY BODY. J.E. Jumblatt and L. O'Connor*. Dept. of Biochemistry and Pharmacology, Tufts Univ. School of Medicine, Boston, MA 02111.

Intraocular pressure in mammalian eyes is regulated by autonomic control of aqueous humor formation and outflow. Although adrenergic drugs are used routinely to treat glaucoma, the cellular mechanisms which underlie their effects on aqueous humor dynamics are complex and somewhat paradoxical. For example, α_1-, α_2- and ß-adrenergic agonists, as well as α- and ß-antagonists, have been reported to lower intraocular pressure in various species. This complexity has been attributed to the multiplicity of postsynaptic adrenergic receptor subtypes thought to exist in ocular blood vessels, ciliary epithelium, smooth muscle and aqueous outflow channels. The possible contribution of presynaptic receptors at autonomic nerve terminals has received little attention. To investigate presynaptic regulatory mechanisms in the rabbit eye, we have studied the effects of endogenous and exogenous mediators on field-stimulated release of ^3H-norepinephrine (^3H-NE) in the isolated, superfused iris-ciliary body.

Iris-ciliary bodies dissected from male albino rabbits were preloaded with ^3H-NE, mounted between two platinum ring electrodes in a temperature-controlled plexiglass chamber, and superfused at 37 °C with buffer containing 1µM cocaine to inhibit neuronal catecholamine reuptake and 3µM indomethacin to inhibit prostaglandin synthesis. Ca^{++}-dependent release of ^3H-NE into the superfusate was evoked by 30 sec trains of pulses at 12 V/cm (10 Hz, 3 msec duration). Test drugs were introduced 5 min before stimulation, and their effects on stimulated (i.e., total minus basal) release were compared to an initial drug-free stimulation of each iris.

Results: (1) α_2-adrenergic agonists strongly depress ^3H-NE release. This inhibition could be overcome by the selective α_2-antagonist yohimbine, which alone facilitated release. Thus α_2-receptor mediated autoinhibition operates in this system. (2) The muscarinic cholinergic agonists acetylcholine (+ eserine) and pilocarpine also inhibited ^3H-NE release. The antagonists atropine and scopolamine alone enhanced release, suggesting that ACh released from parasympathetic neurons may modulate release of ^3H-NE at adrenergic terminals. Stimulation-evoked release of acetylcholine was confirmed by preloading the iris with ^3H-choline and analysis of the release products. (3) No presynaptic effects were seen with ß-agonists, serotonin, morphine, enkephalins, dopamine, histamine, or adenosine at appropriate concentrations. (4) Forskolin, a potent activator of adenylate cyclase, caused a marked enhancement of ^3H-NE release. We are currently exploring the possibility that presynaptic, inhibitory α_2 and muscarinic receptors in the iris-ciliary body may mediate the inhibition of adenylate cyclase. Supported by NEI Grant RO3 EY04487-01

255.1 THE BLOOD-BRAIN BARRIER: CORRELATION BETWEEN MORPHOMETRY AND PERMEABILITY. B.L. Coomber* and P.A. Stewart. Dept. of Anatomy, University of Toronto. Toronto, Ontario CANADA M5S 1A8.

This study examines the correlation between the morphometry of brain capillaries and the regional permeability of the blood-brain barrier (b-bb). Although several morphological differences have been described between barrier and non-barrier capillaries, little quantitative data are available. It is not known, for example, whether increased vesicular formation in permeable areas of the b-bb accounts for their higher permeability, or whether an inter-endothelial route is also necessary. Morphometric parameters of capillaries from gray and white matter of both cerebrum and cerebellum in the mouse were evaluated and compared with those in a permeable area of the brain (area postrema) and with those in muscle. The tissues were fixed by perfusion and examined in the electron microscope. Photographed capillary profiles were measured using computer-assisted planimetry. Non-specific transport across barrier capillaries is restricted by continuous bands of tight junctions and by a low level of vesicular transport as judged by the small number of pinocytotic vesicles observed. We found that vesicle density is uniformly low in gray and white matter endothelia but is 3 times higher in area postrema and 6 times higher in muscle endothelia. Gaps between endothelial cells were never observed in gray and white matter and were only rarely seen in area postrema and muscle. We suggest therefore that the increased permeability of area postrema and muscle vessles is due to increased vesicular transport. Elevated mitochondrial density in barrier vessels is thought to provide energy necessary for maintaining ionic gradients across the barrier. We found an elevated mitochondrial density in cerebral white matter, but not in cerebral gray matter or in cerebellum. Since the b-bb develops later in the cerebellum than in the cerebrum, in the four week old mice the mitochondrial density in the white matter may not have reached adult values. Essential nutrients are transported across the b-bb by membrane-bound transport mechanisms and by diffusion across the endothelial cytoplasm. We hypothesized that the thickness of the endothelial wall may be a rate-limiting factor in this transport, and, therefore, that the walls of barrier capillaries might be thinner than those of non-barrier capillaries. We found that the mean thickness of the endothelial wall is, in fact 50% smaller in cerebral and cerebellar capillaries than in area postrema or muscle capillaries. We suggest that this quantitative approach is useful for evaluating the regional variation in the b-bb both in the developing brain and in the adult brain under normal and pathological conditions.

Personal Support to B.L.C. by Canadian Heart Foundation.
Funded by the Medical Research Council of Canada.

255.2 IS THE BLOOD-BRAIN BARRIER OF INSECTS JUST A SINGLE SEAL OF TIGHT JUNCTIONS, AS IN VERTEBRATES? Stephen R. Shaw, Psychology Dept., Dalhousie University, Halifax, N.S., Canada, B3H 4J1.

Experiments on the insect nerve cord have established that the CNS there is protected by a potent blood-brain barrier (BBB). For many years, the mechanism hypothesized to account for this has been essentially that recognized for the vertebrate CNS: occlusion by a restricted superficial seal of tight junctions connecting up non-neural cells, and confined to the CNS-blood interface (review: Lane, N.J., Int. Rev. Cytol. 73, 243, 1981).

The plausibility of this hypothesis has been tested ultrastructurally on the eyes of Orthoptera and Diptera using extracellular tracers, including lanthanum. The optic lobe contains a barrier that is continuous with the BBB of the nerve cord; the exterior location of the eye facilitates introduction of tracers at different points relative to the putative barrier. Tracer introduced first into the blood penetrates only the outer covering of the CNS, in agreement with earlier work. However, the perineurial cells (supposed site of the BBB in insects) fail to stop the tracer, which comes to rest on the glial layer immediately beneath them. Tracer introduced "behind" the barrier into the eye diffuses freely throughout the retina, despite a recent claim to the contrary. In vivo observations with dyes give a diffusion time of ~15 min per mm for first detection. Tracers also diffuse radially back towards the barrier, but stop abruptly just below the eye's basal lamina, at some distance from the site of arrest when applied to the blood, as found earlier (Shaw, S.R., Cell Tiss. Res. 188, 35, 1978). These results show that the BBB is not a simple, single structure. To test whether it might be a double system made up of two single, separated barriers, tracer has been introduced between the two limits described above. It fails to penetrate detectably beyond a zone of superficial damage, indicating that the barrier property is distributed throughout the tissue.

Several mechanisms cooperate to constitute the barrier. The superficial part is formed by an extreme physical narrowing of the diffusional entry route, by a monolayer of elongate glial cells. Examination of one of the sites of tracer arrest reveals particle rows present on axon membranes along the dominant diffusion route. These are not tight junctions, though they could be some new type of occlusive apposition. True tight junctions are present deep inside the barrier, however, in agreement with a recent report. Finally, both septate junctions and adsorption by the intercellular matrix may form part of the barrier defences. The insect BBB is therefore not present at a single site, does not involve perineurial cells, and appears to be unlike the barrier covering the vertebrate CNS.

Supported by NSERC A9593 and NIH EY04476.

255.3 A MATHEMATICAL MODEL FOR PREDICTING BRAIN CONCENTRATIONS OF THE LARGE NEUTRAL AMINO ACIDS IN HYPERAMMONEMIC RATS. J.H. James*, L.L. Edwards* and J.E. Fischer* (SPON: Alix Mathieu). Dept. of Surgery, Univ. Cincinnati Med. Ctr., Cincinnati, Ohio 45267.

When rats are infused with ammonium salts or are made hyperammonemic by construction of a portacaval anastomosis (PCA), the concentrations of many of the large neutral amino acids (LNAA), particularly phenylalanine, tyrosine and histidine, rise several-fold in the brain. Several reports have shown that the rise in concentration of these amino acids in the brain cannot be accounted for either by their absolute level in plasma or by the ratio in plasma of any one of these amino acids to the sum of its competitors for blood-brain transport. Therefore, some other factor, acting either at the blood-brain barrier or in the brain itself, may alter the transport of the LNAA into the brain. This factor may be related to glutamine, which is synthesized in brain from ammonia and glutamate.

Based on plasma and brain amino acid data from 188 rats which were either infused with ammonium salts and amino acids or underwent a PCA, we have developed a mathematical model which can predict with reasonable accuracy brain concentrations of most of the LNAA from the plasma concentrations of the LNAA and the brain glutamine concentration. The model was developed as follows: (1) The ratio of each LNAA (except tryptophan) to its apparent K_m of transport was calculated both in plasma and in brain, letting glutamine also serve as a competitor with a K_m of transport of 8.5 mM (determined by its ability to competitively inhibit the brain uptake of radiolabeled phenylalanine). (2) The relationship between the calculated ratio (AA/K_m^{app}) in plasma and the same ratio in brain was determined to fit well to a straight line, being near a slope of 1 for amino acids slowly metabolized by brain (phe, his, tyr, met) and around 0.5-0.6 for those which brain can metabolize (leu, ile, val). (3) The eight equations (one for each LNAA - thr, val, met, ile, leu, tyr, phe, his) expressing the relationships between plasma and brain competitor ratios for each amino acid were considered to form a set of eight simultaneous equations in eight unknowns and were rearranged to a form suitable for solution by computer.

Using the series of equations in the model, the brain levels of the LNAA were predicted for the original 188 rats using as input only the plasma LNAA and the brain glutamine level of each rat. The correlation coefficients between the observed and predicted levels were as follows: thr, r=.795; met, r=.838; ile, r=.897; leu, r=.887; val, r=.904; tyr, r=.958; phe, r=.964; his, r=.948. The accuracy of prediction suggests that the primary effect of brain glutamine on the brain levels of other LNAA is as a competitive inhibitor for their efflux from brain.

255.4 STRUCTURAL EVIDENCE FOR UNSTIRRED LAYERS IN THE CHOROID PLEXUS EPITHELIUM. P. A. Grady and O. R. Blaumanis*. Dept. of Neurology, Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

Secretion of cerebrospinal fluid (CSF) by the choroid plexus is an active process in which the pumping of sodium is followed by passive movement of water along an osmotic gradient. A serious dilemma in modeling this system arises from the fact that (a) the sodium pumps are located in the apical membranes of the choroidal epithelial cells which face a large volume of moving fluid, and (b) CSF secretion occurs in the absence of bulk phase osmotic gradients. It has been postulated that local, standing gradients or unstirred layers constitute the osmotic compartment which is required to explain the coupling between sodium and water transport. We present evidence for the existence of the possible structural basis for the postulated sequestered spaces of unstirred layers.

Aldehyde fixed choroid plexus from rats, rabbits and cats was treated with ruthenium red in the presence of osmium. Transmission electron microscopy of the microvilli of choroid epithelial cells revealed dense cell coats on all villi and cilia of these cells. These coats are approximately 20-50 nm thick and appear to be continuous with the outer leaflet of the cell membrane. Neuraminidase, chondroitinase and hyaluronidase digestion diminish or eliminate the staining by ruthenium red, indicating that the coats are composed largely of glycoproteins and are therefore similar to other cell coats. Sections cut perpendicular to the axes of the microvilli showed that the coats of adjacent villi often overlap and are generally in close apposition. Preliminary calculations indicate that the geometry of the microvilli and their thick coats may account for the necessary sequestered space for local osmotic gradients. (Supported in part by PHS Grant #NS16332).

255.5 SIMULTANEOUS DETERMINATION OF REGIONAL BRAIN BLOOD FLOW AND WATER PERMEABILITY BY A DOUBLE-LABEL RADIOISOTOPIC METHOD: INFLUENCE OF STATUS EPILEPTICUS AND HEMISPHERAL NOREPINEPHRINE DEPLETION. M.D. Ginsberg, R.Busto*, S.I. Harik and E. Martinez*. Cerebral Vascular Disease Research Center, Univ. of Miami Sch. of Med., Miami, FL 33101.

We have used a double-label method in the rat to assess regional brain blood flow (rCBF) with (14)C-butanol (a highly extracted diffusible indicator), and permeability with (3)H-water. A mixture of the 2 tracers is infused intravenously over 30 sec, and multiple arterial samples are obtained. The brain is then frozen, and samples are punched from multiple regions of interest. Isotopic activities are computed by a dual-channel internal-standard method. The generalized operational equation derives from Kety: $dCi/dt = mf(Ca - Ci/\lambda)$ (Eq. 1) where Ci and Ca represent tissue and arterial tracer activity; f is rCBF (ml/gm/min), λ is the brain:brain partition coefficient, and m is a diffusion-dependent term for non-ideal tracers. As shown by Crone, $m = 1 - \exp(-PS/f)$. In the present study, a value of m=1 is assumed for the (nearly) ideal tracer butanol. "True" flow f is computed from Eq. 1; the extraction fraction (m) and permeability-times-surface-area product (PS) for water may then be computed.

In Wistar rats ventilated on 100% oxygen, status epilepticus was induced by the IV administration of bicuculline, and secondary hypertension was prevented. After 8 min of seizure activity (by EEG), a radioisotopic study was performed. In one group, norepinephrine (NE) depletion of one cerebral hemisphere had been induced 2 weeks earlier by a 6-hydroxydopamine lesion of the locus ceruleus (LC).

In normocapnic control rats ventilated on 100% oxygen, mean (butanol) flow values (rCBFb) in anterior and posterior neocortex were 2.78±0.78 (SD) ml/gm/min, and mean extraction fraction for water (Ew) was 0.77±0.12. In rats with seizures, rCBFb was 4.08 ±1.17 and 3.89±1.13 ml/gm/min ipsilateral and contralateral to the LC lesion, respectively; and Ew was 0.66±0.09 and 0.67±0.09. Thus, unilateral NE depletion failed to affect either rCBFb or Ew under conditions of intense cortical activation. The regression equations relating Ew to rCBFb in all structures of the 2 animal groups were:

100% O2 CONTROLS: $Ew = -0.056 \times rCBFb + 0.881$, $r = -0.81$

SEIZURE ANIMALS: $Ew = -0.112 \times rCBFb + 1.052$, $r = -0.76$

These 2 equations yield virtually identical values for Ew in their regions of overlapping validity (rCBF range 2-4 ml/gm/min). Thus, brief status epilepticus fails to alter brain water permeability. (Supported by USPHS Grant NS-05820-17.)

255.6 BRAIN ENDOTHELIAL CELL TUBULAR TRANSPORT STRUCTURES ARE NOT FIXATION ARTIFACTS. A.S. Lossinsky*, A.W. Vorbrodt* and H.M. Wisniewski* (SPON: J. Shek). NYS Institute for Basic Research in Developmental Disabilities, Staten Island, N.Y. 10314

Our previous studies of blood-brain barrier (BBB) transport across endothelial cells (ECs) from injured brain cortex showed elongated tubular profiles in addition to pinocytic vesicles. These tubules are thought to serve as conduits for the trans-EC passage of macromolecules because they were shown to transport horseradish peroxidase and ferritin. Further, it was shown that the limiting membranes of both tubules and vesicles showed activity of alkaline phosphatase, an enzyme bound to the plasmalemma of ECs of brain micro-blood vessels. It has also been proposed that the EC tubules (channels) manifest as the result of fusion between adjacent pinocytic vesicles due to improper stabilization by glutaraldehyde of the lipid portion of the plasma membrane. In the present study, we wanted to determine whether or not prefixation of EC plasma membranes with (1) buffered OsO_4 or (2) an osmium-glutaraldehyde coctail would prevent tubular formation. Mice or rats were divided into two groups. Some animals were subjected to crude leptomeningeal injury and perfused fixed initially with either buffered OsO_4, or OsO_4 coctail, followed by glutaraldehyde fixation. These animals were compared to those perfused with glutaraldehyde followed by OsO_4. Fine structural analysis of ECs from both prefixation groups using OsO_4 showed tubular structures similar to the group initially perfused fixed with glutaraldehyde indicating that EC tubules represent transport structures that form in vivo after BBB injury and are not artifacts of fixation.

Financially supported in part by a grant from NINCDS NS18079.

255.7 A COMPARISON BETWEEN THE MICROVASCULAR RESPONSE OF PROXIMAL AND DISTAL CORD SEGMENTS AFTER TRANSECTION. L.J. Noble. Dept. of Anatomy, Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

Spinal cords have been examined 10 min., 1 hr., 3 hrs., 1 day, and 3 days after transection at the T2 vertebral level in the rat. Ultrastructural changes in microvascular permeability proximal and distal to the transection were studied using horseradish peroxidase (HRP) as a vascular tracer. As previously reported (Exp. Neurol., 79:188-211, 1983) a statistically significant increase in pinocytotic activity of HRP was present in sites as remote as 18mm distal to the transection. This response was accompanied by the appearance of reaction product in the vascular basement membrane and cord interstitium from 1 hour through 12 hours post-transection. Similar studies proximal to the surgical site indicate that this impairment of the barrier to HRP persists beyond 12 hours and has been detected 10mm proximal to the transection at 3 days. Despite this evidence of enhanced vascular permeability at later periods the overall microvascular response to HRP appears reduced. Preliminary data demonstrates a characteristic pinocytotic uptake of HRP but often without concomitant leakage into the cord interstitium. Occasional instances of avid pinocytosis of the tracer at the luminal front coincide with intense reaction product in the lumen, suggesting a concentration dependent uptake mechanism. Although the tracer was commonly associated with endothelial vesicles, a majority of these structures remained unlabelled. HRP was also demonstrated in the basement membrane without apparent labelling in the interstitium. However, localization of reaction product in the interstitium may be difficult as it can freely diffuse from abluminal sites. The labelling of the basement membrane in the absence of leakage into the interstitium suggests that it may act as a temporary restraining site for HRP. This research was supported by the Univ. of Maryland Pangborn Fund, 1982-1983.

255.8 ASYMMETRICAL EFFECT OF UNILATERAL CORTICAL SUCTION LESIONS ON RAT CEREBRAL VASCULATURE, R.L. Margolis* and R.G. Robinson, (SPON: E.M. Gruenberg) Dept. of Psychiatry, Johns Hopkins U. Sch. of Med., Baltimore, MD 21205

We have previously demonstrated that a focal lesion of the right frontal cortex in rats produces marked hyperactivity and bilateral catecholamine depletions in the anterior and posterior cortices and in the locus coeruleus. Neither hyperactivity nor catecholamine depletion was produced by identical lesions of the left frontal cortex (Pearlson, G.D., and Robinson, R.G., Brain Res., 218:233, 1981).

To further explore this asymmetry, we examined the effect of focal cortical lesions on cerebral vasculature. Male Sprague-Dawley rats received suction lesions 1.5 mm in diameter in either the left or the right frontal cortex (9.5 mm anterior to ear bar zero). One, five, or fifteen days postoperatively, these rats and controls without lesions were anesthetized and injected intravenously with 15 uCi/Kg ^{14}C-inulin. Seven minutes after injection, when cerebral concentrations had reached a steady state, the rats were sacrificed. Anterior and posterior regions of right and left cortices were dissected using a brain slicing apparatus and assayed for ^{14}C-inulin and protein content.

Analysis of variance revealed a significantly higher anterior ipsilateral ($p < .05$) and posterior contralateral ($p < .003$) cortical inulin content in animals receiving right cortical lesions than in animals receiving left cortical lesions. Differences in inulin levels between the two groups on postoperative days five and fifteen contributed to this effect. The inulin levels of the other two cortical regions examined (posterior ipsilateral and anterior contralateral to the lesion site) were also elevated in rats receiving a right cortical lesion, though not to a statistically significant level.

The pattern of inulin accumulation following unilateral cortical lesions suggests that right and left frontal cortical lesions have different effects on cerebral vasculature. This asymmetry may be related to the depletion of cortical and midbrain catecholamines that is produced by right, but not left, frontal cortical lesions.

255.9 EFFECT OF INTRACAROTID HYPEROSMOLAR MANNITOL ON CEREBRAL CORTICAL ARTERIOLES - A MORPHOMETRIC STUDY. D. W. Beck*, M. N. Hart*, and K. E. Hansen*. (Sponsor: J. C. Godersky). Department of Pathology, The University of Iowa, Iowa City, Iowa 52240

Intracarotid hyperosmolar mannitol infusion has been used as a means to transiently open the blood-brain barrier to proteins and water soluble compounds. The infusion is associated with a transient increase in cerebral blood flow. This study addresses the mechanism of the increased blood flow.

Experiments were performed on 16 male rats weighing between 200 and 220 grams. The rats were anesthetized with pentobarbital and ventilated artificially. Arterial pCO_2, pH, and pO_2 were measured periodically during each experiment and arterial blood pressure was recorded continuously. In nine rats 1.6 M mannitol and 0.9% NaCl was infused into the internal carotid artery via the external carotid artery at a rate of .08 cc/second for 30 seconds. In seven rats 0.9% NaCl alone was infused. Immediately following the infusion the brains were frozen in situ with isopentane cooled in liquid nitrogen, and the wall to lumen (W/L) ratios of the cortical arterioles in the two hemispheres were compared.

Our results show that mannitol causes a decrease in the W/L ratio in all vessels tested compared to controls. For <50μ vessels, W/L was .275 (n = 158) vs. .303 (n = 215) in the opposite hemisphere ($P < .0001$). In >50μ vessels, W/L was .185 (n = 142) vs. .194 (n = 100) ($P > .05$). NaCl infusion alone caused no change in W/L ratio in any sized arterioles.

These results suggest that intracarotid hyperosmolar mannitol causes significant dilation in small cortical arterioles, which may contribute to the transient increase in cerebral blood flow and in addition may contribute to the mechanism for transient opening of the blood-brain barrier.

255.10 MODIFICATION OF BRAIN EXTRACELLULAR SPACE BY CO_2: NEURAL REGULATION. T. A. Kent*, G. Nagy*, A. Oke, S. Preskorn, R. N. Adams (SPON: M. K. Shellenberger). Depts. of Psychiatry and Pharmacology, Univ. of Kansas Medical Center, Kansas City, KS 66103 and Dept. of Chemistry, Univ. of Kansas, Lawrence, KS.

Several groups have reported that increasing cerebral blood flow (CBF) increases the brain capillary permeability and surface area product (PS) of a variety of compounds. The single pass PS to the diffusion-limited compound, water, increases with CO_2, a relationship which is altered by adrenergic manipulation (Preskorn, et al, Science, 1981). In this study, we examined whether this PS change reflects an alteration in the brain microenvironment. We measured the effect of CO_2 on brain extracellular space (ECS) by means of an ion selective electrode sensitive to alpha-naphthalene sulfonate (ANS). This compound has been used as a marker of ECS (Nicholson & Phillips, Neurosci. Abst, 1981). Rodents were anesthetized (chloral hydrate, 400 mg/kg i.p.), intubated, and passively ventilated with N_2 (55-70%), O_2 (30%), and CO_2 (0-15%). Two glass pipettes were implanted into the thalamus, and Na-ANS was introduced via a nanoliter pump through one pipette. The pipette used to measure ANS concentration changes was filled with a PVC-based ion exchanger mixture containing Aliquat (Aldrich)/Na-ANS as active material. CO_2 produced a dose dependent decrease in local concentration of ANS, indicating an increase in its volume of distribution, consistent with a 10-20% increase in ECS. This effect reversed when CO_2 was stopped. Moreover, lidocaine (2%, 5-8 ul) injected into the brainstem in the region of the locus coeruleus reversibly decreased the CO_2 effect by ca. 50%. These results suggest that CO_2 expands ECS, presumably by a transfer of water and/or other solutes from the intravascular space, although intracellular shifts cannot be ruled out. Moreover, a neural contribution to the effect, probably by the central adrenergic system, is suggested. These CO_2 effects differ from Cameron, et al, (Yale J. Biol. Med., 1969) who found no change in the volume of distribution of ^{14}C-sucrose in brain as measured by ventriculocisternal perfusion. Possible reasons for this difference, such as pH or blood flow artifacts, are being investigated. We are also currently examining the effects of amitriptyline--a tricyclic antidepressant and indirect adrenergic agonist, known to increase PS to water--on the CO_2-induced increase in ECS. This data may aid in development of strategies employing low doses of CO_2 and adrenergic agonists to increase the brain concentration of poorly diffusible chemotherapeutic agents.

255.11 INFLUENCE OF PENTYLENETETRAZOLE INDUCED SEIZURES ON 86-RUBIDIUM TRANSPORT IN CEREBRAL MICROVESSELS, K.M.A. Welch, J.A. Helpern and S.J. McGee*. Dept. of Neurology, Henry Ford Hospital, Detroit, MI 48202.

Techniques for the isolation of metabolically active cerebral microvessels (MV) are well established (Goldstein GW, et al, J Neurochem, 25:715, 1975). The isolated MV allows for the study of the blood brain barrier in vitro. Previous studies from this laboratory have shown that pentylenetetrazole (PTZ) induced seizures in gerbils increased 2-deoxyglucose uptake when compared to control (Nell JH, Welch KMA, Ann Neurol, 7:457, 1980). The presence of Na^+-K^+ ATPase dependent K^+ (as 86-Rubidium) uptake in isolated cerebral MV has previously been documented (Eisenberg HM and Suddith RL, Science, 206:1083, 1979; Chaplin ER, et al, Biochem Pharm, 30:241, 1981). We now demonstrate an effect of PTZ induced seizures on 86-Rubidium (86-Rb^+) uptake in isolated cerebral MV. Sprague-Dawley rats were injected with PTZ (IP, 150 mg/kg) and allowed to seize for 1 minute after onset of clonic seizures. Another group of animals were allowed to seize until death. MV were isolated from the cortical shells, which had been dissected free of white matter. Isolated MV were incubated in 1.0 ml of Hepes buffered Ringers (pH=7.4) containing 2.5 μCi/ml of 86-Rb^+ for 0,2,4,6 and 8 minutes. Uptake was stopped by the addition of 3 ml of ice cold buffer. The MV were then washed 3 times with 3 ml each of ice cold buffer. The radioactivity remaining in the MV was determined by liquid scintillation and the data normalized per mg protein. MV isolated from animals 1 minute after the onset of clonic seizures showed no increase in uptake of 86-Rb^+ when compared to control. In contrast, MV isolated from animals allowed to seize until death showed a significant ($p< 0.05$) increase in 86-Rb^+ uptake. This increase in uptake over control was not inhibited by the addition of oubain. We conclude that the increased uptake of 86-Rb^+ into isolated MV, after prolonged seizure, reflects a passive diffusion (leakage) of K^+ through an impaired blood brain barrier and not active transport.

255.12 ASYMMETRICAL EFFECT OF CEREBRAL INFARCTION ON DISTRIBUTION VOLUMES FOR C-14 INULIN IN THE RAT CORTEX. R.S. Black*, and R.G. Robinson (Spon: Scott Lukas) Dept. of Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

We have previously described the asymmetrical effect of ischemic lesions of rat frontal cortex on behavior and brain catecholamines. Right middle cerebral artery ligation led to behavioral hyperactivity, and bilateral 20-40% depletions in cortical and midbrain norepinephrine with complete or partial return to control levels over a 40 day postoperative period. Left-sided lesions, however, produced no such changes (Robinson, Science, 205:707-710, 1979). To further investigate this asymmetry we have used intravenous infusion of radiolabeled inulin to quantitate the effects of these lesions on the cerebral vasculature.

The right or left middle cerebral artery of 300-350 g rats was visualized through a fronto-parietal craniotomy, ligated with an 8-0 silk suture and severed. The lesion produced involved only the cortex and resulted in no postoperative mortality or lasting neurologic deficits. C-14 radiolabeled inulin (New England Nuclear) was injected intravenously at 15 uCi/kg at 1, 5, and 15 days postoperatively. Seven minutes after injection rats were sacrificed and 3 mm wide anterior and posterior full thickness samples of cortex were dissected using a mechanical brain slicing apparatus. Brain slices were assayed for protein and C-14 inulin content.

In animals sacrificed one day after surgery both right and left anterior ipsilateral sample groups (those containing the lesion) had inulin counts higher than control (t-test,$p<.001$); at 5 days only the right anterior ipsilateral group remained elevated compared to control ($p<.001$); and at 15 days all sample groups were significantly below the control levels ($p<.05$). Analysis of variance revealed significantly higher inulin content ($p<.05$) in the anterior ipsilateral cortex of right sided animals compared to left. Individual means were significantly different on day 5 with right greater than left in anterior ipsilateral and contralateral groups ($p<.05$), with comparisons in the posterior cortex near significant ($p<.1$). By day 15, however, samples from left lesioned animals had consistently higher counts than right, though not significant. No right-left differences in lesion size or protein content (tissue quantity) were detected. Asymmetries in inulin space between right and left lesion animals paralleled asymmetries in cortical and midbrain catecholamine deficits seen in earlier experiments, suggesting a relationship between the biochemical and vascular consequences of ischemic injury in this model.

255.13 DIFFERENTIAL EFFECTS OF CARNITINE, OCTANOYLCARNITINE AND OCTANOIC ACID ON THE UPTAKE OF 5-HYDROXYINDOLEACETIC ACID BY RABBIT CHOROID PLEXUS. C.S. Kim* and C.R. Roe* (SPON: J.F. Howard). Dept. of Neurology and Biol. Sci. Res. Ctr., Univ. of North Carolina Sch. of Med., Chapel Hill, NC 27514 and Dept. of Pediatrics, Duke Univ. Med. Ctr., Durham, NC 27706.

The roles of carnitine in fatty acid metabolism include (1) facilitated transport of acyl compounds into mitochondria and (2) acylcarnitines are one of the primary forms transporting in plasma. The pathogenesis of brain encephalopathy involves the production of free fatty acids in the brain and these acids play an important role in mitochondrial damage and subsequent cerebral energy failure. Carnitine deficiency and increased fatty acids are common metabolic disorders in Reye's syndrome. The present *in vitro* study was undertaken to determine whether carnitine and acylcarnitine have any protective role on the transport of endogenous organic acid, 5-hydroxyindoleacetic acid (5-HIAA).

Choroid plexus (CP) was collected from the lateral and fourth ventricles (LVCP,FVCP) and incubated for either 10 min or 60 min in artificial CSF containing ^{14}C-HIAA. When the incubation medium contained either 0.1mM or 1.0mM octanoic acid, the uptake of ^{14}C-HIAA at 10 min was reduced in a dose related manner (37% and 53% in FVCP; 48% and 67% in LVCP, respectively). After 60 min incubation, T/M ratio of 4.1 ± 0.4 (S.E.) and 6.0 ± 0.6 were achieved in FVCP and LVCP in control media. The ratios were decreased to 2.5 ± 0.2 and 2.8 ± 0.2 in media containing 1.0mM octanoic acid (P<0.001). With the same levels of exposure to octanoylcarnitine, the uptake was inhibited only at higher concentration (1.0mM) by 36% and 43% in FVCP and LVCP, respectively, at 10 min incubation. The prolonged incubation for 60 min abolished the inhibitory effect of octanoylcarnitine on uptake completely. However, treatment with carnitine at the same concentration did not inhibit ^{14}C-HIAA uptake by either FVCP or LVCP. Carnitine actually appeared to stimulate the uptake at higher concentration (1.0mM) during both 10 min and 60 min incubation.

We have previously shown that the 5-HIAA transport mechanism shares the choroidal carrier for other organic acids. The present study demonstrates that carnitine and octanoylcarnitine in contrast to octanoic acid improve the uptake of 5-HIAA by choroid plexus through a mechanism known to be a major route for excretion from the brain *in vivo*. Carnitine may be a useful therapeutic adjunct for removal of toxic organic acids from the CNS in Reye's Syndrome.

255.14 EFFECT OF ACETAZOLAMIDE AND OUABAIN ON CEREBRAL ZINC METABOLISM IN RATS. E. J. Kasarskis and D. Walls*. Dept. Neurology, VA and Univ. Kentucky Med. Ctrs., Sanders-Brown Aging Ctr., Lexington, KY 40536.

Previous studies from this laboratory have demonstrated a slow rate of entry and turnover of zinc (Zn) in rat brain following pulse administration of ^{65}Zn (Soc Neurosci Abstr 7:87,1981). In contrast to neural tissue, the choroid plexus avidly accumulated ^{65}Zn suggesting that the choroid plexus may function in regulating cerebral Zn homeostasis. In order to test this hypothesis, ouabain and acetazolamide, drugs known to affect the secretory and absorptive function of the choroid plexus, were administered to determine if these drugs altered cerebral Zn metabolism.

Twenty adult male Sprague-Dawley rats received a single intraperitoneal dose of either ouabain (10 mg/Kg), acetazolamide (100 mg/Kg) or saline vehicle followed 15 min later by intravenous ^{65}Zn [25 μCi/Kg in 0.1 mL saline] under thiopental anesthesia. Rats were sacrificed after 6 hrs; samples of plasma, CSF, lateral ventricle choroid plexus, arachnoid, eye, and brain (subsequently dissected into 7 regions) were obtained for assay of ^{65}Zn content according to our previously-described methods (Soc Neurosci Abstr 7:87,1981). In addition, samples of liver, kidney, and muscle were taken in order to assess the effect of these drugs on systemic, extracerebral Zn metabolism.

Acetazolamide increased ^{65}Zn concentration 3-fold in CSF despite lowering plasma ^{65}Zn to 75% of control but did not affect the content of ^{65}Zn in the choroid plexus itself. The level of ^{65}Zn was elevated between 1.3 and 1.5-fold in all brain regions due to acetazolamide. In contrast, ouabain decreased ^{65}Zn concentration in brain regions to 90% of controls. Both drugs significantly decreased the Zn content of eye, arachnoid, and kidney but neither drug affected Zn metabolism in liver or muscle.

These data suggest that the carbonic anhydrase inhibitor, acetazolamide, can acutely alter Zn metabolism in rat brain causing significant elevations of newly administered ^{65}Zn. Although the mechanism underlying this effect is unknown, inhibition of choroidal secretion of CSF by acetazolamide and reduction of CSF turnover may retard intracranial elimination of Zn via CSF outflow pathways, causing a "backing up" of ^{65}Zn in extracellular fluid of brain. Alternatively, the choroid plexus could function by transporting Zn from CSF back into plasma as has been demonstrated for calcium (J Neurosci 2:1322,1982). Further studies are needed to evaluate these hypotheses. (Supported by VA Research Service and TIDA grant, NS 00768).

255.15 RADIATION INDUCED BBB BREAKDOWN IN RATS: THE THRESHOLD? M. P. Remler and W. Marcussen*, Dept. of Neurology, VAMC, Univ. of California, Davis, Martinez, CA, 94553

Ionizing radiation is known to lower the blood brain barrier (BBB) (Remler, M.P., Exp. Neurol. 73:310, 1981). The threshold of this effect for tissue has been confused by varying species, volumes of tissue radiated and techniques for monitoring the change in BBB function. The mechanism for the delayed BBB breakdown remains unclear. Using systemic convulsants, it is possible to detect very small BBB lesions (Remler, M.P., Acta neurol. scand. 65:51, 1982).

Bicuculline methiodide (Bm), the GABA blocking epileptogen, crosses the normal BBB of rats poorly (Pong, S.F. Brain Res. 42:486, 1972) and produces no consistent abnormality behaviorally or on EEG at 6.0 mg./kg. (n=10). The BBB opened in 0.25 cu. cm. of cortex by alpha particles. The 33 out of 33 rats radiated were clinically normal. When challenged with 6.0 mg./kg. Bm. 2 out of 11 showed some scattered sharp waves and 1, presumably with accidental cortical trauma, prominant spike activity. When 6,000r are given and then the Bm this results in an intense highly localized epileptiform discharge which begins about 20 minutes post injection and lasts 30 to 90 minutes in 18 out of 19 rats. This result is reproducible for the 1 week the BBB is open after irradiation. The radiation degredation of the BBB is detectable as low as 4,000r.

The probability of microfluctuations of background radiation reaching toxic levels to individual cells is discussed. The role of exposure of the brain to otherwise sequestered chemicals and the release of sequestered brain antigens into the circulation as mechanisms of delayed toxicity will be discussed.

255.16 VASOPRESSIN CROSSES THE BLOOD-CSF BARRIER IN BRATTLEBORO RATS. J.M. Lyness*, C.D. Sladek, and D.M. Gash (SPON: S.J. Wiegand). Departments of Anatomy and Neurology, University of Rochester, Rochester, NY 14642.

The permeability of the blood-cerebrospinal fluid (CSF) barrier to vasopressin was examined in the homozygous Brattleboro rat, which congenitally lacks vasopressin. The effects of chronic infusion were studied by the use of intraperitoneally implanted osmotic minipumps (Alza, Palo Alto, CA) set to deliver 1 μl/hr of physiological saline vehicle. Controls (n=10) received only saline; experimental animals received 0.1 μg/hr (n=5), 1.0 μg/hr (n=5), or 5.0 μg/hr (n=4) arginine vasopressin (AVP, Bachem, Torrance, CA). Three days after pump implantation the animals were anesthetized (Chloropent, .3 ml/kg b.w.); blood samples were obtained for serum AVP radioimmunoassay (RIA) by cardiac puncture, and blood-free CSF samples, obtained from the cisterna magna, were pooled for AVP RIA. Similar procedures were used on acutely infused animals, which were taken one hour after intraperitoneal injection of saline (n=6) or 0.1 μg (n=6) 1.0 μg (n=5), or 5.0 μg (n=6) AVP.

The results show that higher levels of AVP, chronically or acutely infused, do cross the blood-CSF barrier. In both chronic and acute cases, saline controls had very low serum levels of cross-reacting substance (measuring ≤ 1.0 pg/ml), and undetectable levels in the CSF (≤1.0 pg/ml). The 0.1 μg groups had average serum AVP levels of 12.8 pg/ml with chronic infusions and 6.8 pg/ml with acute infusions; CSF levels were 1.1 pg/ml and undetectable (≤1.0 pg/ml), respectively. The 1.0 μg chronic group had 68 pg/ml in the serum and 58 pg/ml in the CSF; the 1.0 μg acute group had a serum level of 70.5 pg/ml and a CSF level of 34 pg/ml. The 5.0 μg animals had extremely high serum AVP levels (≥ 160 pg/ml chronically and ≥ 400 pg/ml acutely) but only slightly higher CSF levels than the 1.0 μg groups (64 pg/ml and 81.4 pg/ml, respectively).

These results suggest that the AVP found in normal rat CSF is secreted into the CSF independently of systemic release, since at the 0.1 μg level, which produced serum AVP levels in the high physiological range, AVP did not cross the blood-CSF barrier in appreciable quantity. However, the permeability of the barrier at higher levels indicates that the behavioral effects of systemically injected AVP could be due to central effects of the peptide. Why AVP can cross the barrier at higher levels is unknown; the possibility that the peptide's hypertensive effects at high levels may disrupt the barrier and alter its permeability to AVP is currently being investigated.

Supported by grant No. NS15109 and AM19761.

255.17 Na/H EXCHANGE IN THE RAT CHOROID PLEXUS (CP). Vincent A. Murphy* and Conrad E. Johanson. Dept. of Pharmacology, Univ. of Utah, Salt Lake City, UT 84132.

The functional relationship between Na transport and the $pH/pCO_2/HCO_3$ system is a fundamental, but poorly understood problem in electrolyte physiology. Na/H exchange transport has been demonstrated in various systems: small intestine, kidney proximal tubule, gall bladder, gastric parietal cell, and skeletal muscle. Demonstration of H gradient-dependent Na uptake is one way to show the existence of this system in a tissue.

Male adult rats were used in all studies. Animals were nephrectomized under ether anesthesia prior to agent and isotope treatment. Either NaCl, $NaHCO_3$, NH_4Cl, or HCl 4.7 mmol/kg; acetazolamide 25 mg/kg; or vehicle was given IP to alter the transmembrane H gradient. One hour post nephrectomy the rats were anesthetized with ketamine 80 mg/kg IP and plasma, CSF, and lateral and fourth ventricle CP's were obtained as samples. Isotopes were administered as follows: i) ^{14}C-dimethadione and ^{3}H-raffinose, 0 min. post treatment; ii) ^{22}Na, 0, 12, 24, 36, 42, or 48 min. post treatment; and iii) ^{51}Cr-RBC, 50 min. post treatment. The dimethadione, raffinose, and RBC were used to determine CSF/CP cell pH, extracellular fluid volume, and residual erythrocyte volume respectively. Na and K were measured by first extracting with 0.02 N HNO_3/0.015 N Li, then using flame photometry. Isotopes were counted by either a Beckman Biogamma or LS 7500. Na uptake was determined by either taking the slope of the volume of distribution (V_d) vs. time (CSF) or by curve stripping using the stable Na V_d as steady state (SS), ln (SS - V_d) vs. time (CP).

A strong correlation between the basolateral H gradient (blood side) and Na uptake into the CP was found (r=0.99); the basolateral HCO_3 gradient also correlated but not as well (r=0.90). CSF uptake of Na also correlated with CP uptake indicating that a basolateral system is being affected. Other parameters such as plasma, CSF, and CP cell [Na], [K], [H], and [HCO_3] correlated less significantly ($r < 0.85$) with ^{22}Na uptake.

The results demonstrate that much of the Na uptake into the CP is due to Na/H exchange transport and possibly Na/HCO_3 cotransport. This permits a further understanding of the relationship between H/HCO_3 and Na uptake in the CSF/CP system. (Supported by NIH grant NS 13988.)

255.18 TRANSPORT KINETICS OF LARGE NEUTRAL AMINO ACIDS ACROSS THE BLOOD-BRAIN BARRIER. Y. Takasato*, Q.R. Smith and S.I. Rapoport. Lab. of Neurosciences, Gerontology Research Center, National Institute on Aging, Baltimore City Hospitals, Baltimore, MD 21224.

To characterize the transport kinetics of large neutral amino acids at the blood-brain barrier, we measured the concentration-dependent uptakes of tryptophan, leucine, isoleucine and cycloleucine, using an in vivo brain perfusion technique. In pentobarbital-anesthetized rats, the right pterygopalatine artery was ligated and the external carotid artery was catheterized. Just after ligation of the common carotid artery, the right cerebral hemisphere was perfused by retrograde infusion (0.083 ml/s) of a physiological saline (37° C, pH 7.4) into the external carotid artery. During perfusion, circulating blood contributed less than 3% to capillary fluid flow in the right cerebral hemisphere. The capillary fluid flow in the cerebral cortex, as determined by ^{57}Co-labeled microspheres, was $1.17 \pm 0.05 \times 10^{-1}$ s^{-1}. The perfusion fluid contained ^{3}H-inulin, ^{14}C-labeled amino acid and various concentrations of unlabeled amino acid (0-20 mM). After 5 to 60 s of perfusion, the rat was decapitated and samples from 6 brain regions and infusion fluid were analyzed for radiotracer content. The cerebrovascular permeability-area product (PA) was calculated from the brain parenchymal concentraction of tracer (C*br), perfusion fluid tracer concentration (C*pf), perfusion time (T) and brain capillary fluid flow (F); PA = -F.ln (1- C*br/F .C*pf.T). For each amino acid, Vmax and Km for the saturable component of uptake and Kd for nonsaturable uptake were determined from nonlinear regression analysis; PA = Vmax/(Km + Cpf) + Kd, where Cpf = amino acid concentration.

In the parietal cerebral cortex, PA for each amino acid decreased approximately 100 fold when Cpf was elevated from 0 to 20 mM. Calculated Vmax ranged from $0.93 \pm 0.07 \times 10^{-3}$ µmol/s/g for cycloleucine to $1.05 \pm 0.08 \times 10^{-3}$ for isoleucine. In contrast to this relative constancy of Vmax, Km (mM) was 0.010 ± 0.004 for tryptophan, 0.024 ± 0.006 for leucine, 0.057 ± 0.007 for isoleucine and 0.280 ± 0.040 for cycloleucine. A nonsaturable component of uptake (Kd) was not detected for isoleucine or cycloleucine, whereas, for both tryptophan and leucine, Kd was significantly greater than zero and approximated PA for passive diffusion, as predicted by the octanol/water partition coefficient. Lastly, significant regional differences in Vmax, Km and Kd were not observed among the 6 perfused brain regions. These data show that large neutral amino acids cross the blood-brain barrier predominantly by carrier-mediated transport, and that the carrier affinity for the amino acids is greater than previously thought.

255.19 BRAIN UPTAKE OF ERYTHROSIN B, A FOOD DYE, PREVENTED BY BINDING TO PLASMA PROTEIN IN RATS. H. Levitan, Q.R. Smith, Y. Takasato,* S.I. Rapoport, and Z. Ziylan*, Lab. of Neuroscience, GRC, National Institute on Aging, Baltimore, MD 21224.

Food colors have been held responsible for several behavioral disorders such as minimal brain dysfunction and hyperactivity. Some dyes affect neuronal function when directly applied. There is no information however on whether significant quantities of the food coloring dyes enter the brain after consumption or parenteral administration. To determine if a widely-used food dye, erythrosin B (FD&C Red No. 3), can cross the blood-brain barrier (BBB) and enter the brain, we administered ^{14}C-erythrosin B directly into the circulation of mature (3 month-old), male, Osborne-Mendel rats, and measured radioactivity in brain regions at several times thereafter. We found that ^{14}C-erythrosin B did not enter the brain from the blood, but penetrated the BBB when infused directly into the carotid circulation in the absence of blood protein. Under pentobarbital anesthesia, the right external carotid artery of a rat was cannulated distal to its junction with the common carotid, and the right common carotid artery was encircled with thread. The cannula was connected to a syringe containing ^{14}C-erythrosin B (0.1-0.2 µCi/ml, spec. act. 630 mCi/mmol) and ^{3}H-inulin (0.6-1.1 µCi/ml, spec. act. 1750 mCi/mmol) in an oxygenated and warmed bicarbonate Ringer's solution (4 experiments) or in heparinized rat whole blood (4 experiments). The common carotid artery was ligated, and Ringer's solution or whole blood was infused immediately into the external carotid artery. The animal was decapitated after 60 s and the brain dissected into six regions (frontal lobe, parietal lobe, occipital lobe, hippocampus, caudate nucleus, thalamus-hypothalamus). Net brain ^{14}C-radioactivity was corrected for residual intravascular tracer by subtracting the radioactivity attributable to inulin, which does not cross the blood-brain barrier. Significant parenchemal concentrations of ^{14}C-erythrosin B were detected in all brain regions when it was perfused with protein-free Ringers. The cerebrovascular permeability-surface area product (PA) ranged from $6\text{-}7 \times 10^{-4}$ s^{-1}, about 300x the PA of sucrose, as predicted from the octanol-water partition coefficient of the dye. Insignificant parenchymal radioactivity was detected in brains infused with dye in whole blood. From equilibrium dialysis, about 99.7% of the dye was shown to be bound to plasma protein. The results show that, although free erythrosin B can rapidly cross the BBB, signficant brain uptake of intravascular dye is prevented by its binding to plasma protein, and by blood-brain barrier iimpermeability to the dye-protein complex. Sensitivity to food dyes such as erythrosin B in some individuals may reflect altered plasma protein binding capacity, which can vary with disease and age.

259 SYMPOSIUM. POST-TRANSLATIONAL PROCESSING OF NEUROPEPTIDE PRECURSORS AND NEURONAL PROTEINS. H. Gainer, NICHD, NIH (Chairman); R. Hammerschlag, City of Hope, Nat'l Med. Center; Y. P. Loh, NICHD, NIH; L.D. Fricker, Johns Hopkins Univ.; C. Glembotski, Univ. of Penn.; N.A. Ingoglia, New Jersey College of Medicine.

It has become apparent in recent years that the phenotypic expression of a gene product in the nervous system and other tissues depends on more than transcriptional and translational processes. Many post-translational modifications of the newly synthesized protein may be necessary before these molecules become functionally active. In addition, such processing mechanisms can serve to activate or inactivate a peptide or protein either reversibly or irreversibly. Among these mechanisms are glycosylation, sulfation, phosphorylation, acetylation, amidation, aminoacylation, methylation, ADP-ribosylation, and limited proteolysis. Biologically active neuropeptides are subjected to many of these processes, and examples of these mechanisms will be described here for the ACTH-endorphin and enkephalin precursor proteins. The emergence of α-MSH and endorphin from the ACTH-endorphin precursor first requires the hydrolysis of specific peptide bonds in the precursor by limited proteolysis (at loci in the precursor which contain pairs of basic amino acids, eg. Lys-Arg). This is followed by further limited proteolysis (eg. carboxypeptidase-B-like enzyme action), and N-acetylation and amidation of the peptide products. All of these mechanisms are necessary to produce the appropriate peptide structure for biological activity (or inactivity, in the case of N-acetylated endorphin). The intracellular organization of post-translational processing is a highly relevant issue and will be discussed both for neuropeptide biosynthesis and fast axonally transported (i.e., neuronal membrane) proteins. The phenomenon of axonal transport in neurons provides an experimental window through which the subcellular localizations of some of these processes can be examined, and the mechanisms of glycosylation and sulfation will be addressed in this context. Although it is well known that axons and nerve terminals do not synthesize proteins de novo, recent evidence indicates an abundance of transfer-RNA in axoplasm. A potential post-translational processing role for this t-RNA, and a possible function for aminoacylation in growth and regeneration in axons will be discussed.

260 SYMPOSIUM. EFFERENT CONTROL OF THE ORGANS OF HEARING AND EQUILIBRIUM. S.M. Highstein, Albert Einstein College of Medicine (Chairman); J.M. Goldberg, University of Chicago; J.C. Adams, Medical University of South Carolina; J.J. Guinan, Massachusetts Eye and Ear Infirmary; J. Art*, University of Cambridge.

Using a multidisciplinary approach, the central nervous system control of the organs of hearing and equilibrium is being studied in squirrel monkeys, cats, turtles and fish. Efferent control of the lateral line organs is inhibitory and is thought to prevent self excitation of exteroceptors. In the cat, auditory efferents have been divided into "lateral" and "medial" groups of cells. Axons of lateral neurons are myelinated and project to the inner hair cell region bilaterally (mostly ipsi) and medial neurons (unmyelinated) project to the outer hair cell region bilaterally (mostly contra). The morphology suggests that most of the results from electrical stimulation of crossed and uncrossed olivo-cochlear fibers are due to stimulation of myelinated fibers from the medial subgroups and not to activation of unmyelinated fibers from lateral cells. Further, extensive double labeling experiments with retrograde tracers and cholinesterase staining demonstrate central collaterals of both the auditory and vestibular efferents. The auditory cells have terminal plexes restricted to the anterior ventral cochlear nucleus and to the dorsal pole of the dorsal cochlear nucleus. Vestibular efferents have central terminal plexes beneath the entire extent of the olivo-cochlear bundle from below the MLF to the facial nerve genu, in the interstitial nucleus of the VIIIth nerve and in the ventro-lateral vestibular nucleus. Intracellular records taken from turtle hair cells indicate that electrical stimulation of efferents generates hyperpolarizing synaptic potentials and a concomitant loss of tonal sensitivity and tuning. Ion substitution experiments suggest that the hyperpolarization is mediated mainly by an increase in the hair cell membrane permeability to potassium. Dr. D.O. Kim has been invited to discuss the biomechanical effects of efferent activation on the organ of Corti.

In squirrel monkeys, vestibular primary afferent activity is increased by electrical stimulation of the efferent vestibular system. Irregularly discharging afferents are 10-20X more sensitive than regularly discharging afferents. The efferent vestibular system may function to extend the dynamic range of the afferents. In toadfish, efferent vestibular neurons are separated into distinct subgroups on cytoarchitectural grounds. They have central axon collaterals in the MLF, and branch centrally to innervate more than one peripheral endorgan. Intracellular recording demonstrates certain patterns of electrical coupling among various subgroups. Efferent neurons increase their discharge frequency when the fish is roused to move. The speakers will attempt a unified hypothesis for the physiology of these efferent systems.

OPIATES III

261.1 OPIATE WITHDRAWAL BEHAVIOR AFTER PROLONGED FOCAL BRAIN STIMULATION IN THE RAT. B. E. Thorn-Gray and D. A. Williams*. Dept. of Psychology, The Ohio State University, Columbus, OH 43210.

Focal electrical stimulation of the periaqueductal gray (PAG) produces analgesia in rats. This analgesia is assumed to be partially mediated by the endogenous opiates since the phenomenon is attenuated by Naloxone. The analgesic effect of stimulation-produced analgesia (SPA) is behaviorally similar to the effects observed after morphine administration. If morphine is administered for a prolonged period of time, opiate withdrawal behaviors (indicative of opiate dependence) are observed after administration of Naloxone. This study investigates whether Naloxone-elicited opiate withdrawal behaviors result from prolonged SPA.

Twelve albino rats were surgically implanted with one bipolar stimulating electrode into the PAG. The tail-flick technique was used to assess analgesia. Withdrawal behavior was measured by injecting the rat with Naloxone (IP, 1 mg/kg), placing it in an observation box, and checking for the presence of opiate withdrawal behaviors as delineated in published opiate withdrawal scales.

Each rat, serving as its own control, was exposed to three conditions: Condition I (control) - No stimulation-Naloxone-Withdrawal Test, Condition II (control) - 2 hr. confinement in stimulation tubes (no stimulation)-Naloxone-Withdrawal Test, Condition III (SPA Trial) - Stimulation (10" of stimulation every 2' for 2 hr.)-Naloxone-Withdrawal Test. (Parameters: freq. 50 hz., intensity 20-100 μa, pulse duration 1 μsec.)

Compared to the control conditions, after two hours of SPA and Naloxone administration, rats exhibited a significantly greater frequency of opiate withdrawal behaviors ($Q = 6.17$, $p < .05$). Additionally, the mean number of withdrawal behavioral categories increased from $\bar{X} = 2.5$ for controls to $\bar{X} = 6.3$ following the SPA condition ($Q = 9.08$, $p < .05$).

The withdrawal behaviors following the SPA trial were behaviors usually associated with a low grade dependence on morphine. This finding suggests an association between prolonged SPA and low grade opiate dependence in rats.

261.2 THE NEUROCHEMICAL MECHANISM FOR STIMULATION-PRODUCED ANALGESIA: COMPARISON OF TESTS INVOLVING TONIC AND PHASIC PAIN. K.E. Plotkin* and B.E. Thorn-Gray (SPON: D. Meyer) Lab of Physiological and Comparative Psychology, Ohio State University, Columbus, OH 43210

The hypothesis that different types of pain may be mediated by separate neural subsystems has been extended by Ronald Melzack and others. Melzack suggests that pain measures such as the Tail-Flick Test primarily engage a group of fibers (lateral pain-signalling system) that are responsible for the communication of transient stimuli. Conversely, the Formalin Test involves the injury to tissue (via a .05 ml injection of 5% Formalin solution) on the dorsal surface of a rat's forepaw, imposing a constant, inescapable pain stimulus. A large component of the pain resulting from tissue damage may be tonic in nature, and transmitted by a more medial set of fibers. These neural subsystems may be modulated separately, and may serve discreet purposes in the integrative aspects of pain response. This experiment investigated the possibility that different neurotransmitters are involved in stimulation-produced analgesia as measured by the Tail-Flick and Formalin Tests.

Male albino rats were implanted with bipolar electrodes in the periaqueductal gray. During the procedure of each pain test, analgesia from focal brain stimulation (Parameters: freq. 50 hz., intensity 20-50 μa, pulse duration 1 μsec.) was challenged with either IP naloxone (3 mg/kg) or methysergide (1 mg/kg).

Using the Tail-Flick Test, both naloxone and methysergide reduced analgesia or eliminated it. Using the Formalin test naloxone failed to reverse stimulation-produced analgesia; mean pain ratings remained at analgesic values after the opiate antagonist was administered. However, methysergide reversed the analgesia significantly; mean pain ratings increased to 70% of baseline values after its administration. The data suggest that a non-opioid system is involved in the mediation of tonic pain.

261.3 ANALGESIC POTENTIATION AND THE DISTRIBUTION OF MORPHINE IN THE BRAIN IN THE PRESENCE OF TRIPLENNAMINE IN MICE. R. Bluhm*, M.A. Evans, and E.K. Zsigmond. Lab. of University of Illinois at Chicago, Chicago, IL 60612.

Interest in the interaction of opioids and histamine antagonists arose from the observation that abusers of pentazocine and triplennamine experience a heroin-like euphoria that neither drug alone produces. The present study was conducted to evaluate the analgesic properties of the combination of the histamine antagonist, triplennamine (TRP), and morphine (MS). Analgesia was assessed in male, Swiss Webster mice weighing 25-35 gm using the hot plate at 55°C and all drugs were administered intravenously. TRP had no analgesic effect. When a sub-optimal dose of MS (3.5 mg/kg) was combined with TRP in a dosage range of 2.5-5 mg/kg the latency in response to pain 10 minutes after drug administration was significantly increased relative to morphine alone. Values for the three treatment groups were 1.79±0.36sec., 7.07±0.71, and 24.39±2.02 ($p \leq 0.02$), respectively, for TRP (5 mg/kg), MS, and MS coadministered with TRP. This potentiation was also evident 30 minutes after drug administration when the latency in animals receiving the drug combination was still significantly longer. Motor coordination was not affected. Naloxone completely reversed the analgesic response of animals receiving MS and the MS-TRP combination.

The distribution of (N-methyl-^{3}H) morphine (30 μCi/kg) in the presence of TRP (4 mg/kg) was studied. Regional brain areas and plasma were sampled and dissolved in Soluene-350^R (Packard Instruments) before being counted for radioactivity. The levels of radioactivity in the striatum as expressed by tissue: plasma ratios for MS and MS-TRP respectively after 10 minutes were 3.2±0.4 and 1.9±0.2. After 30 minutes, the tissue to plasma ratio was 1.03±0.04 and 1.45±0.05 for MS and MS-TRP respectively. In the cerebral cortex the corresponding values were 0.58±.05, 0.67±0.1 for 10min samples and 0.50±0.03, 0.55±0.02 for the 30min samples. Similarly, the distribution of radioactivity in the hypothalamus, brain stem and plasma was not significantly different in the presence and absence of TRP.

The mechanism of the analgesic potentiation of MS by TRP does not appear to be due to changes in morphine distribution in plasma or in the brain.

261.4 NOCICEPTION IN DEVELOPING RATS. David M. Bronstein*, Mina Mir Mohammed Sadeghi* and Loy D. Lytle (SPON: R. W. Reynolds). Laboratory of Psychopharmacology, Department of Psychology, University of California, Santa Barbara, CA 93106.

Although there has been a great deal of research investigating the neuroanatomical and neurochemical mechanisms which mediate the pain responses of adult animals, relatively little is known about the development of nociception in those animals born immature. The purpose of the present study was to characterize the responses of rats of different ages following the intraperitoneal injection of a noxious hypertonic saline solution.

Groups of 55 male or female albino rats, bred in our colony from Sprague-Dawley derived rats obtained from Simonsen Laboratories (Gilroy, CA), were tested for 5 consecutive days beginning at ages 8, 13, 18, 28, 38, or 48 days following birth. All testing took place during the latter half of the light phase of the light:dark cycle. The testing procedure involved injecting each animal intraperitoneally with 1 ml/kg of one of 6 concentrations (3, 4, 8, 12, 16, or 20%) of hypertonic saline, and then observing them for the frequency and duration of a writhing response (a response in which animals show waves of rostral to caudal contractions of the abdominal musculature, and a stretching and/or arching of the back). The test session was terminated when an animal emitted no writhing response for a continuous 60 sec period.

In general, animals younger than 40 days of age showed a decreased responsivity/sensitivity to the noxious hypertonic saline treatment. For any given dose of saline, approximately 3 times as many animals that were 20 days of age or older emitted writhing responses following injection compared to 10 day old rats. Furthermore, the intensity of the writhing response increased both as a function of the age of the animal tested, as well as a function of the dose of hypertonic saline administered at any given age. Thus, the number of writhing responses an animal made and the total time an animal spent emitting the writhing responses gradually increased with age, until adult-like patterns were attained by 40 days of age. Further studies are planned to determine whether age-dependent changes in responsivity/sensitivity to hypertonic saline injections observed in this study might generalize to other noxious stimuli. However, our data thus far indicate that immature animals do not have fully mature, functional neuroanatomical and/or neurochemical mechanisms important for nociception. (Supported in part by NIMH grant MH-31134).

261.5 LEU-ENKEPHALIN EFFECTS ON ACQUISITION AND CONSOLIDATION OF AN ACTIVE AVOIDANCE TASK, R.C. Dana*, P. Conner* and J.L. Martinez, Jr., Dept. of Psychobiol., Univ. of Calif., Irvine, CA 92717. (SPON: J. A. Kusske)

Previously we reported that opioids are potent modulators of avoidance responding when administered prior to training (Neurosci. Abs.,1980,6:319). The purpose of these studies was to compare the effects of Leu-enkephalin (LE) on an active avoidance task when administered at different times relative to training.

Male Swiss-Webster mice (27-35 gm) were trained and tested in a two-compartment, trough-shaped alleyway. A white safe compartment was separated by a sliding door from a black footshock (0.8 mA) compartment. Mice were placed in the dark compartment facing away from the door. If the animal failed to cross to the safe compartment within 10 sec, the shock was turned on and terminated when the mouse escaped. 4 trials were run on Day 1, with a new trial being initiated every 30 sec. 24 trials were run on Day 2, and 10 trials were run on Day 5 with 15 sec to shock onset. Mice were injected with LE(200 ug/kg,I.P.), LE plus naloxone (Nx,200 mg/kg, I.P.) or saline (SAL) immediately after training on Day 1 (Expt. 1), or 1 min prior to training on Day 2 (Expt. 2).

MEAN AVOIDANCE RESPONSES, ± S.E.M., (n)

		Day 2	Day 5
Expt. 1	SAL	9.1 ± 4.3 (12)	4.8 ± 2.8 (15)
treatment on	LE	1.3 ± 2.0 (12)[a,b]	1.5 ± 1.8 (14)[a]
Day 1	LE + Nx	4.8 ± 5.2 (12)	2.9 ± 3.1 (7)
Expt. 2	SAL	8.8 ± 6.0 (29)	2.2 ± 2.5 (12)
treatment on	LE	3.7 ± 5.0 (26)[a]	1.0 ± 1.1 (10)
Day 2	LE + Nx	5.2 ± 5.2 (14)[a]	3.1 ± 1.5 (7)[b]

[a]Sig. diff from SAL, [b]Sig. diff. from LE, Dunnett's procedure.

In Expt. 1 LE impaired consolidation of the training experienced on Day 1. Animals treated with LE following training on Day 1 also exhibited impaired learning on Day 2 and Day 5 indicating that LE produces long-term effects. LE and NX given after training on Day 1 attenuated, but did not block the effect observed on Day 2. However, by Day 5 the LE + Nx group was indistinguishable from SAL. In Expt. 2 LE given before training impaired acquisition of the avoidance task, but the effect was not evident on Day 5 as it was in Expt. 1. Nx attenuated the effect of LE given before training on Day 2, but in contrast to Expt. 1, by Day 5 there was a clear cut antgonism of the LE effect.

LE was shown to impair acquisition and consolidation of an avoidance task. A high dose of Nx attenuated the actions of LE, particularly in the consolidation paradigm. Finally, the effect of LE given following training was long-term and was evident several days following administration. (Supported by ONR Contract N00014-82-K-0385 to J.L.M.)

261.6 BEHAVIORAL SIMILARITIES BETWEEN NALOXONE-PRECIPITATED MORPHINE WITHDRAWAL AND LOCUS COERULEUS STIMULATION IN MONKEYS. S. J. Grant, D. E. Redmond, Jr. Primate Research Laboratory. Yale School of Medicine, New Haven, Ct 06510.

Convergent evidence suggests that central noradrenergic systems contribute to the development of many of the physiological and behavioral manifestations of morphine withdrawal. However, behaviors elicited by stimulation of the noradrenergic nucleus locus coeruleus (LC) have not been directly compared with morphine withdrawal effects under identical conditions. In the present study, behaviors observed during naloxone-precipitated morphine withdrawal in chair-restrained Macaca arctoides were compared with behaviors elicited by electrical field stimulation of the LC.

Three female monkeys implanted with bilateral LC electrodes received continuous unilateral biphasic electrical field stimulation of the LC (50 Hz, 0.4-1.2 mAmp, 0.5 msec pulse width) for 30 minutes. Four female monkeys were addicted to morphine via subcutaneous slow release morphine pellets, while 3 female monkeys served as controls. Opiate withdrawal was precipitated by naloxone injections (0.002-0.016 mg/kg, I. M.) spaced at least 72 hours apart. Analysis of variance or covariance was used to evaluate the statistical significance of results.

LC stimulation produced a significant increase in the same restricted set of behaviors reported previously, without increases in general activity or distress behaviors. LC-related behaviors included oral movements, self-directed movements (e.g. hair and skin pulling), grasping of the chair, and jerky body movements.

Naloxone administration in the morphine-addicted monkeys increased the LC-related behaviors in a highly dose-dependent manner, but general activity did not increase significantly until the 0.016 mg/kg dose. Sedation and freezing behaviors which increased during morphine administration declined following naloxone injections. Control monkeys did not exhibit significant changes in any of the behaviors following naloxone administration.

Thus, behaviors associated with LC stimulation also increase during naloxone-precipitated morphine withdrawal. However, LC-stimulation produces a more specific increase in certain behaviors, while morphine withdrawal appears to produce a more general behavioral activation. These data are consistent with and suggest a behavioral consequence of the reported interactions of opioids with the LC at the molecular, intracellular, and cellular level (Redmond, D.E., Jr., editor, J. Clin. Psychiat. 43(6):1-48, 1982).

Supported in part by U.S.P.H.S. Grants DA02321, MH31176, the Harry Frank Guggenheim Foundation, and R.S.C.D.A. DA-00075 to D.E.R.

261.7 NALOXONAZINE: DOSE-DEPENDENT AND TEST-DEPENDENT EFFECTS UPON MORPHINE ANALGESIA. D. A. Simone,* R. J. Bodnar, and G. W. Pasternak. Dept. of Psychology, Queens College, CUNY, Flushing, NY and George C. Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Dept. of Neurology and Pharmacology, Cornell University Medical Center, New York, NY.

The long-lasting opioid antagonist naloxonazine (NAZ), competes equally well with both endogenous and exogenous opioids for all opiate receptor sub-types for at least 48h. Morphine analgesia is eliminated by NAZ (10mg/kg) intravenously administered 24h prior to the opiate. The present study evaluated the dose-response relationships of intracerebroventricular (icv) administration of NAZ upon basal pain thresholds as well as morphine analgesia 24h following NAZ. Groups of six male albino Sprague-Dawley rats were matched according to baseline tail-flick latencies followed by jump thresholds which were determined over a four day period. Each group received only one NAZ dose (0, 1, 5, 50μg/10ul, icv) followed 24h later by one of the following morphine doses (5, 10, 15mg/kg, sc). Tail-flick latencies and jump thresholds were determined at 0.5, 1, 2, and 24h after NAZ administration and 0.5, 1, and 2h after morphine administration. An additional group received naloxone (50μg/10ul, icv) 24h prior to a morphine dose of 10mg/kg NAZ failed to alter either basal tail-flick latencies or basal jump thresholds at any post-injection interval. However, NAZ exhibited dose-dependent and test-dependent effects upon morphine analgesia. While all NAZ doses significantly attenuated the elevations in jump thresholds 0.5 and 1h after a 15mg/kg dose of morphine, only the 50μg NAZ dose was capable of significantly reducing morphine's (15mg/kg) effects on the tail-flick test. A similar antagonistic relationship of all NAZ doses upon morphine (10mg/kg) analgesia was observed with jump thresholds significantly reduced at all time points and tail-flick latencies altered by only the 50μg NAZ dose. Following 5mg/kg of morphine, elevations in jump thresholds were eliminated by NAZ (1 and 50μg) while again, only the 50μg NAZ dose significantly reduced tail flick latencies.

These date are discussed in terms of NAZ's long-term ability to antagonize opiate analgesia through opiate receptor blockade, the central site of action for such effects and the differential effects as a function of the pain test employed. (Supported by NIH grants GRSG 5S05RR07064 and DA02615).

261.8 LONG-TERM ADMINISTRATION OF MORPHINE REDUCES ADIPOSITY IN RATS N. D. Courtney, A. L. Riley, R. A. Gach* and S. C. Woods. Dept. of Psychology, Univ. of Washington, Seattle, WA 98195.

After starvation, when fat stores are depleted, rats will selectively increase dietary fat intake. Chronic morphine injection also leads to a selective increase in fat consumption by rats. Since it has been reported that endogenous opiods stimulate lipolysis, this suggests that the selective appetite for dietary fat in morphine-treated rats may be due to a morphine-stimulated loss of adipose stores. The present experiment was designed to determine the effects of chronic morphine injection on adipose tissue in rats.

Ninety-day-old Long Evans female rats (n=12) were divided into two groups. The first group received morphine sulfate (intraperitoneal 40 mg/kg) injections and the second group received equivolume injections of saline for 22 days. Both groups were given ad libitum access to standard Purina rat chow and water. Ovarian (OV) and retroperitoneal (RP) fat pad weights, fat cell numbers and lipoprotein lipase (LPL) levels were determined on the 23rd day.

	Morphine	Saline
BODY WEIGHT	237.2(8.1)	244.3(9.0)
FAT PAD WEIGHT (g)		
OV:	0.61(0.10)**	1.71(0.10)
RP:	0.27(0.03)**	0.73(0.04)
LPL (nmoles FFA/10^6cells/min)		
OV:	83.2(16.1)	82.0(18.5)
RP:	58.3(10.5)	51.6(9.0)

** =P <.05(t-test) mean(SEM)

Both fat pad weights were significantly decreased in the morphine treated rats compared to the controls, whereas LPL levels and body weights did not differ significantly.

This decrease in adipose tissue weight with no change in LPL levels in morphine treated rats, indicates that the reduced adiposity may not be due to decreased ability to store fat. It is possible that this decrease in adiposity is due to morphine induced stimulation of lipolysis. The lack of change in body weight with a concomitant decrease in adiposity with morphine injections, suggests a shift in caloric deposition from adipose tissue to lean body mass.

261.9 NEUROBEHAVIORAL RESPONSES OF INTACT AND CAUDATE NUCLEI LESIONED CATS DURING A CYCLE OF ADDICTION TO LOW DOSES OF MORPHINE. J.R. Villablanca, C.M. Harris, W. Burgess and I. De Andrés, Ment. Ret. Res. Ctr., Depts. Psychiatry & Anat., UCLA Sch. Med., LA, CA 90024

After 2 week baseline rectal temperature (RT) and body weight measurements, 5 intact and 5 cats with bilateral caudate nuclei lesions (acaudates) received a 12 day course of 3 mg/kg/day,i.p. morphine sulphate (MS), with 3 animals in each group continuing until 16 days. On day 12 and 16 respectively, the cats received 1.0 mg/kg i.v. naloxone (NX) 2½ hr after MS; NX alone was repeated 15-30 days after this first precipitated withdrawal. On days 1,5,11 and 15 and during NX days cats were placed in a one-way mirror, sound attenuated chamber for 2 hr prior and 5 hrs after MS (or 2 hrs after NX). Using a described video time-sampling procedure, the percentage time per sample and the per sample frequency of the following posture-movements were determined: walking, standing, sitting, crouching, lying down, discrete head and paw movements. Other items below were assessed from video samples or direct observations. RT was measured at the end of video sessions and 45 min after NX. Vomiting and weight were recorded daily. Intact cats showed little changes in above behaviors. Acaudates showed reduction of walking (26.5%) and increase in lying (67.1%) and head movements (16.2%), all non-significant, suggesting tolerance to activating effects. Sleep or drowsiness were not seen. There was tolerance to hyperthermia (intact p<.009, acaudates p<.02); vomiting was idiosyncratic to each cat and weight decreased (p<.01) during the withdrawal month for only acaudates. At the end of MS course all animals appeared slowed down, distressed, groomed poorly, vocalized plaintively and often salivated when handled but gross behavioral manifestations of caudate lesion (Exp. Neurol. 52: 389; 1978) were still present. After NX on the last day of MS, for about 10 min all cats appeared agitated (moving briskly, attempting to escape, backing, rearing, etc.) except for one acaudate (largest ablation) who stood immobile and developed pronounced catatonia; they showed panting, salivation, photosensitivity, urinated or sprayed, vocalized plaintively and some showed catatonic-like posturing for over 30 mins. For about 30 min, there were "wet-dog" body or head shakes. After 10-15 min cats calmed down, sat or crouched, but tachypnea continued for 60-100 min such that all cats slept only after that. Mydriasis and hyperthermia were blocked and, in a testing at 45 min, no signs of acaudate approaching syndrome were seen. Following the late NX injection, cats showed panting (N:2) or tachypnea (N:6) for about 20 min; most showed head shakes and fewer body shakes, vocalization, licking, mild activation and tail posturing. After saline injection, only sporadic vocalization and mild escape behavior were seen. In brief, the outstanding early and protracted withdrawal contrasted with mild signs of tolerance seen. (Grants DA-02518 & HD-04612).

261.10 LOW DOSES OF MORPHINE DEPRESS AFFECTIVE BEHAVIOR SCORES IN CATS. C.M. Harris and J.R. Villablanca. Mental Retardation Res. Ctr., Depts. Psychiatry and Anatomy, UCLA Sch. Med., LA, CA 90024

Feline affect has previously been studied with regard to reward and punishment related behavior. However, to detect drug-induced affective changes in cats not subjected to any strongly aversive or rewarding stimuli, we designed a test in which spontaneous and rater-elicited actions of freely roaming cats were observed. Nine mongrel adult male cats (3 intact, 6 brain-lesioned) were tested before, during and after a 16-day course of chronic morphine sulphate (MS), 3 mg/kg/day, ip. This dose is below that required for mania (see companion Abstract). The lesions included one cat with removal of one cerebral hemisphere, two with unilateral, and three with bilateral lesions of the caudate nucleus. The test battery included a checklist of 20 behaviors such as hiding and spontaneous approach, and a 7-item rating scale which indicated the rater's impression of states such as happy/sad and violent/gentle. Observations were made in a vivarium containing several racks of caged cats, a few dark niches and a large open-floor area. The rater refrained from interacting with the cat for 20 min while recording spontaneous activity. About 5 min were then devoted to interactions in which the rater called to the cat and engaged in petting and gentle play. If the cat did not approach when called, the rater spent about 5 min attempting actively to elicit purring and treading. The test battery was administered several times during the baseline periods, before and after chronic MS, and both before and 3 hrs after the injection on days 2, 7 and 14 of the treatment Paired comparisons between pre- and post-injection scores during the treatment and between pre- and post-treatment baseline scores were carried out with the Wilcoxon Signed Rank test. No striking differences were found among scores generated by five independent raters, two of whom were blind to the subjects' drug status Early in the course of treatment MS caused a significant lowering of test scores (p<.014, day 2), which suggests that affect was lowered by MS. This result is similar to the finding that, in humans, observer ratings indicate MS-induced mood-depression and decreased friendliness, although subjects report euphoria (Psychopharmacol. 75:124, 1981). Scores dropped each time the drug was administered and returned to pretreatment levels within 24 hrs. A comparison of change scores on days 2 and 14 indicated no tolerance to this effect, however tolerance may depend on surgical status, as the intact cats appeared to become more sensitive while the lesioned cats became less sensitive to the drug effect. A comparison of pre-treatment and post-withdrawal baseline scores indicated that no permanent change resulted from chronic treatment. (Supported by USPHS Grants DA-02518 and HD-04612).

261.11 SLEEP-WAKEFULNESS EFFECT OF SINGLE DOSES OF MORPHINE IN CATS. I. de Andrés and J. R. Villablanca, Dept. Morfología, Fac. Medicina, Univ. Autónoma, Madrid, Spain. Mental Retardation Research Ctr., Depts. Psychiatry and Anat. UCLA Sch. of Med. L.A., CA 90024

This report is a part of a continuing study of the neurobehavioral actions of low doses of morphine in intact and brain lesioned cats (Brain Res., 248: 159; 1982). Seven adult cats were implanted with neocortical and pontine, orbital and neck muscles standard electrodes to record EEG, EOG and EMG respectively. After recovering for 15 days or longer, four animals received i. p. injections of morphine sulfate at the dose of .5, 1.0 and 2.0 mg/kg. Each dose was administered only once per animal and the interval between injections was at least 15 days. The other 3 animals received a single injection of 3.0 mg/kg. All cats were continously recorded in a sound-attenuated chamber with one-way mirror for 1 hr. before and 72 hr. after morphine administration. The records were scored according to stablished poligraphic features defining wakefulness (W), drowsiness (D), NREM and REM sleep (S). Saline vehicle injections using each cat as its own control, were used for comparisons (Wilcoxon test) of the hourly values for the four S-W states during each daily cycle.

After morphine, both NREMs and REMs were suppressed for a time period which correlated with dose: NREMs, range=6-15 hr., r=0.63 $p<0.05$, REMs, range=9-25 hr., r=0.84, $p<0.01$. Thereafter, NREMs increased significantly starting by the 6th to 15th post-morphine hr. of the first day. REMs levels were restored but not rebound was seen. NREMs rebound continued through the second day, REMS values were non-significantly difference from controls, and W together with D remained significantly reduced (except for D at .5 mg/kg). Third day values for all stages were equivalent to controls, except for NREMs which, at the 3.0 mg/kg dose, stayed significantly increased for the first 6-7 hr. of that day.

In summary, there was a dose-dependent suppression of sleep with a protracted rebound for NREMs but not for REMs. In the context of our previous results in diencephalic and caudate nucleus lesioned cats, an interaction between brain stem and forebrain processed is proposed to understand S-W, and some behavioral effects of acute morphine administration in the cat (Supported by FISSS, Spain, Grant 51881 and USPHS Grants DA-02518 and HD-04612).

261.12 MORPHINE AND NALOXONE: EFFECTS ON THE DETECTION OF NON-REWARDING BRAIN STIMULATION. J.E.G. Williams*, K.R. Green*, H.S. Wheeling* and C. Kornetsky (SPON: D. Sax). Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118

Previous experiments in our laboratory demonstrated that morphine raises (Marcus, R. and Kornetsky, C. Psychopharmacology, 38: 1-13, 1974) while naloxone lowers (Sasson, S. and Kornetsky, C. Pharmacol. Biochem. and Behav., 18:231-233, 1983) the threshold for aversive electrical stimulation to the mesencephalic reticular formation. In order to determine whether or not these threshold changes are due to actions on a pain system rather than alterations in attentional or perceptual behavior, the effects of both morphine and naloxone on the detection of non-aversive stimulation to this same midbrain site were determined. The threshold level of stimulation for detection as employed in this procedure is by itself neither positively nor negatively reinforcing; however, this level of stimulation can be used as a discriminative stimulus in a simple instrumental task.

Male albino rats (CDF - Charles River Laboratories) were stereotaxically implanted with bipolar stainless steel electrodes aimed at the mesencephalic reticular formation (MRF) and the medial forebrain bundle - lateral hypothalamic area (MFB-LH). Following surgery, the animals were trained to make an instrumental response, a quarter turn of a wheel manipulandum, to a 0.5 sec MRF stimulation cue (S1). Responding to the cue within 7.5 sec was maintained by the delivery of a reinforcing contingent stimulus (S2) to the MFB-LH area. Absolute detection thresholds were determined by varying the current intensity of the brain stimulation cue (S1) according to a modification of the psychophysical method of constant stimuli. The contingent stimulus (S2) remained at a fixed highly rewarding intensity level. Preliminary results indicate that neither morphine (4.0-12.0 mg/kg) nor naloxone (4.0-16.0 mg/kg) will alter the detection threshold at doses that significantly raise or lower, respectively, the escape threshold for aversive stimulation to the MRF. These results suggest that these previously reported effects are not due to actions on attentional or perceptual systems but are due to direct effects on a pain system.

(Supported in part by NIDA grant DA 02326).

261.13 MORPHINE AND d-AMPHETAMINE: EFFECTS ON BRAIN-STIMULATION REWARD. C.B. Hubner*, G.T. Bain* and C. Kornetsky (SPON: R. Feldman). Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.

d-Amphetamine is often self administered with opiates in order to maximize its euphorigenic effects. Also, it has been shown that d-amphetamine potentiates the analgesic efficacy of morphine in man (Forrest, W.H., et al., New Engl. J. Med., 296(13):712-715, 1977) and the rat (Sasson, S., et al., Abstracts Society Neurosciences, 1983). Increased sensitivity for rewarding brain stimulation has been used as an animal model for drug-induced euphoria and is thought to be predictive of abuse liability. Individually, morphine and d-amphetamine at appropriate doses have been shown to significantly lower brain-stimulation reward thresholds (Marcus, R. and Kornetsky, C., Psychopharmacologia, 38: 1-13, 1974; Esposito, R.U., et al., Psychopharmacology, 69:187-191, 1980). In the present study doses of morphine and d-amphetamine which by themselves caused non-significant or just significant changes in threshold were tested together. Given the street use of d-amphetamine in combination with opiate drugs it would be expected that the results would reveal a synergistic action.

Male albino rats (CDF - Charles River Laboratories) were stereotaxically implanted with bipolar stainless steel electrodes aimed at the medial forebrain bundle. Determination of the brain-stimulation reward thresholds was accomplished by using a variation of the psychophysical method of limits. Various doses of morphine (0.125-4.0 mg/kg sc) and d-amphetamine (0.03-0.5 mg/kg ip) were tested separately in each animal. Ineffective or minimally effective doses of both morphine and d-amphetamine were then selected for each animal and tested in combination. The results indicate that minimally effective doses of morphine when combined with ineffective doses of d-amphetamine caused a lowering of the reward threshold far in excess of what has been observed by either drug at any dose. This synergistic effect is congruent with the reported effects of street use of the combination and probably contributes to the reported potentiation of the analgesic effect of morphine by d-amphetamine.

(Supported in part by NIDA grant DA 02326).

261.PO SUDDEN DEATH, ACUTE AND CHRONIC EXTRAPYRAMIDAL SYNDROMES INCLUDING TARDIVE DYSKINESIA AND SELF-MUTILATION INDUCED BY FLUPHENAZINE AND HALOPERIDOL IN MONKEYS. E.F. Domino, Dept. of Pharmacology, Univ. of Mich., Ann Arbor 48109 and Lafayette Clinic, Detroit, MI 48207.

For a number of years our laboratory has been studying various species of monkeys given fluphenazine and/or haloperidol in an attempt to develop a subhuman primate model of tardive dyskinesia. It has been possible to induce a syndrome of tardive dyskinesia in Cebus apella which we have reported previously (Kovacic and Domino, 1982, 1983). In the process of studying Macaca speciosa, several other syndromes were observed which are the subject of this report. A total of 8 adult animals, 3 males and 5 females, were studied. All of the animals were in excellent physical and neurological condition prior to neuroleptic treatment. Thus, any brain damage that might have been sustained because of their previous research history was minimal, if any. Each animal was given first 25 mg of fluphenazine decanoate and later the enanthate (3.2 mg/kg) i.m. every two weeks and 5 days a week haloperidol first i.m. and later orally. Haloperidol was given first in doses of 1.0 mg/kg and after several years of therapy reached 6.4 mg/kg/day. All of the animals developed severe bradykinesia and related acute extrapyramidal side effects. Gradually, over a period of months to years, these effects diminished so that larger doses of haloperidol were tolerated. Animals who survived gained weight. Three of the monkeys (Fifi, Pete and Sam) completed 3 to 5 years of uninterrupted neuroleptic treatment. Three (Babe, Bette and Brandy) died suddenly after 1 to 3 weeks of neuroleptic treatment. Two (Ben and Carol) developed self-mutilation syndromes after several months of neuroleptic therapy. These syndromes were so destructive (eating off fingers, chewing on testicles, chewing on the arms sufficient to expose bone, etc.) that neuroleptic treatment was discontinued for humane reasons. The three animals which completed several years of uninterrupted neuroleptic treatment never showed any tardive dyskinesia while on this medication. When neuroleptic treatment was stopped abruptly, there was an increase in alertness and loss of the bradykinesia and tremor. Within one month, two of the three monkeys showed an increase in mouthing and chewing movements which disappeared in Fifi in about two weeks. On the other hand, Sam developed marked mouthing and chewing movements, marked akathisia of the hands and feet which peaked in about 3 months and gradually diminished over a period of about one year after neuroleptic withdrawal. It is concluded that neuroleptics can induce in Macaca speciosa syndromes very similar to those described in man in addition to a self-mutilation syndrome which is clearly drug induced. Although Macaca speciosa in captivity are known to self-mutilate without any drugs, chronic neuroleptic medication dramatically enhances such behavior in some animals.

262.1 MORPHOLOGY OF PHYSIOLOGICALLY IDENTIFIED GANGLION CELLS IN THE CAT RETINA. L.R. Stanford and S.M. Sherman. Dept. of Neurobiology & Behavior, SUNY at Stony Brook, NY 11794.

Response properties of single cat retinal ganglion cells recorded in vivo via a transcleral approach have allowed us to classify these neurons as W-, X-, or Y-cells based on a battery of physiological tests. These tests included response latency to antidromic stimulation of the optic chiasm and tract, linearity of spatial and temporal summation, size and position of receptive field, type of receptive field (ON or OFF center/surround, direction selective, etc.), briskness of response, sustained or transient center response, and response to fast-moving (>200°/sec) targets. After electrophysiological testing and classification, the ganglion cells were impaled, re-classified, and iontophoretically injected with HRP. Following perfusion, the dissected retina was flat-mounted and reacted using the cobalt-intensified DAB protocol to demonstrate the HRP reaction product. This provided a Golgi-like filling of the soma, dendrites, and axon of each cell. To date, 15 ganglion cells have been successfully injected and recovered. These include 2 Y-cells, 7 X-cells, and 6 W-cells. Based on the morphological criteria of earlier Golgi studies, the 2 recovered Y-cells have been identified as alpha cells, having thick axons, large somata, and large dendritic fields. Of the 7 X-cells recovered, all but 1 had soma sizes and dendritic architecture previously ascribed to the beta cell class. The other X-cell had a soma size within the beta cell range but had dendrites that were similar to those previously described for gamma cells. All of our X-cells had medium-sized axons. Our injected W-cells showed the most variability in both soma size and dendritic architecture. These cells had thin axons and included some neurons with soma sizes within the previously described gamma cell range but others with somata within the size range of beta cells. All of our recovered W-cells had thinner dendrites and sparser dendritic fields than did our recovered X-cells. However, when compared with X-cells, the W-cell dendritic fields were more variable in size, and their soma positions were often eccentrically placed within the dendritic fields. Our results thus far support and extend the general structure/function correlations proposed by Wassle and colleagues.

Supported by USPHS Grants EY04080 and EY03080.

262.2 IDENTIFICATION OF CELL TYPES IN RAT RETINA USING MONOCLONAL ANTIBODIES. C.J. Barnstable, K. Akagawa* and R. Hofstein.* Department of Neurobiology, Harvard Medical School, Boston MA and The Rockefeller University, 1230 York Avenue, New York.

We can label each major subclass of rat retinal cells by monoclonal antibodies raised against neuronal tissues.

Rod photoreceptors can be labelled with the previously described antibodies RET-P1, RET-P2 and RET-P3 as well as monoclonal antibodies raised against purified bovine rhodopsin such as antibody RHO-C7 (a gift from M. Applebury).

We have cloned two antibodies, RET-B1 and RET-B2, that label primarily bipolar cells. From the number of cells labelled and their terminal patterns in the inner plexiform layer it is likely that these antibodies are reacting with both ON and OFF bipolars. These antibodies also label the inner segments only of photoreceptors and a few cells at the inner edge of the inner nuclear layer.

Ganglion cells are the only cells in retina to express the cell surface antigen Thy-1. They can also be labelled with antibodies against neurofilaments.

A-type horizontal cells are the only other major cell class to be labelled with neurofilament antibodies. In addition these cells can be labelled with some antibodies that react with cytoplasmic antigens of glial cells -- making them unusual in expressing both glial and neuronal cell markers.

HPC-1, an antibody raised against rat hippocampus, selectively labels the amacrine cell layer and a few cell bodies in the ganglion cell layers. From double-labelling experiments with neurofilament antisera, the cells in the ganglion cell layer seem to be displaced amacrine cells.

The major glial cells in retina, Muller cells, can be labelled by a variety of membrane antibodies, RET-G1 - G6, most of which are not found elsewhere in the CNS. In addition we have produced a number of antibodies against intermediate filament proteins selective for Muller cells (and A-type horizontal cells).

One antibody, produced by in vitro immunisation of naive spleen cells, has been found that in retina is selective for the astrocytes of the optic nerve fibre layer.

These and other antibodies have been used to examine the postnatal development of the rat retina in terms of the expression of the individual cell-type specific antigens.

(Supported by NIH grants EY 03735, NS 17309 and a grant from the Hereditary Disease Foundation.)

262.3 PUTATIVE SYNAPTIC CYTOSKELETAL COMPONENTS IN RETINA. S. D. Flanagan and B. Yost*. Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

Insolubility in non-denaturing detergents is an excellent operational means for the initial identification of cellular cytoskeletal components. When the cerebral membranes are subjected to the action of the bile detergent deoxycholate (DOC), two proteins, which have previously been identified as possible molecular components of postsynaptic densities (PSDs), are the predominant Coomassie blue staining species observed upon SDS-PAGE of the DOC-insoluble fraction (Flanagan, S.D. et al., J. Cell Biol., 94:743, 1982). One of the proteins, tubulin, has been localized to the PSD by immunocytochemical localization techniques, but is also a ubiquitous component of nonsynaptic microtubular structures. The second is a 51,000 M_r calmodulin binding protein, which has been co-purified in subcellular fractions enriched in PSDs.

The retina contains synapses that are morphologically homologous to those observed in the cerebrum as well as structures apparently unique to the inter- and outer-plexiform layers, e.g., ribbon synapses. If the DOC-insoluble fraction indeed reflects authentic synaptic cytoskeletal components, then apparent morphological differences in synaptic structures may be reflected in cerebral versus retinal DOC-insoluble fractions. Although the yields of DOC-insoluble protein from bovine retina and rat cerebral membranes were comparable (5.2% from bovine retina and 6.7% of the total membrane protein from rat cerebrum), there were several apparent differences in their protein compositions. The 51,000 M_r region on SDS-PAGE gels constituted only 2% of the retinal DOC-insoluble fraction based upon staining by Coomassie blue, as compared with 16% for cerebrum. The 51,000 M_r cerebral protein, a prominent calmodulin binding protein, may be visualized by overlay of SDS-PAGE gels with ^{125}I-calmodulin and subsequent autoradiography. In order to quantify the low levels of this protein in retina, we improved techniques for detection of calmodulin binding proteins, allowing the quantification of as little as 15 ng of the cerebral 51,000 M_r DOC-insoluble protein. This technique revealed a detectable level of 51,000 M_r calmodulin binding protein in both crude and DOC-insoluble fractions from retina, but at levels 30 to 35-fold lower than the comparable cerebral fractions. Other differences in the DOC-insoluble fractions from retina and cerebrum were the higher intensity of two proteins of 32,000 M_r and 43,000 M_r (actin or a closely related protein) in fractions from retina when compared with cerebrum.

262.4 STARBURST AMACRINE CELLS: INTERNUNCIAL CHOLINERGIC NEURONS SELECTIVE for ON and OFF PATHWAYS TO RETINAL GANGLION CELLS. E. V. Famiglietti, Jr. Dept. of Anatomy, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

Starburst amacrine cells in rabbit retina have been demonstrated to have the salient morphological features of cholinergic retinal neurons which have been demonstrated autoradiographically by Masland and Mills ('79). Type a starburst amacrine cells have cell bodies in the amacrine cell layer and narrow dendritic stratification in sublamina a of the inner plexiform layer (IPL), while type b cells have cell bodies in the ganglion cell layer and stratification in the middle of sublamina b of the IPL (Famiglietti, '83).

By electron microscopy of serially sectioned, Golgi-impregnated cells, type a (OFF) and type b (ON) starburst amacrine cells are shown to have qualitatively the same synaptic connections: input from cone bipolar cells and from amacrine cells, and output exclusively to dendrites of ganglion cells.

Apart from mirror-symmetrical distribution, morphological differences between type a and type b starburst amacrine cells are slight. Asymmetry exists, however, in the synaptic input from bipolar and amacrine cells. In particular, type a starburst amacrine cells have more amacrine input than type b cells. Since a heavily labelled band of GAD (glutamic acid decarboxylase) positive synaptic terminals coincides with the substratum of type a starburst amacrine cells, but no corresponding heavily labelled band overlaps the substratum of type b starburst amacrine cells (cf. Brandon et. al., '81; Famiglietti and Vaughn, unpubl.), it is likely that a significant fraction of the input to starburst amacrine cells is GABAergic. Studies are underway to confirm this proposal, and to identify the morphological and functional types of ganglion cells which are postsynaptic to starburst amacrine cells.

(Supported by NIH grant no. EY 03547)

262.5 THE SHAPE OF THE CHOLINERGIC AMACRINE CELLS IN THE RABBIT RETINA: IDENTIFICATION BY INJECTING FIXED, FLUORESCENT NEURONS. Masaki Tauchi* and Richard Masland, Harvard Medical School, Boston MA.

A difficulty in studying the inner retina is the heterogeneity of the amacrine and ganglion cell populations. Depending on the method of classification, most mammalian retinas probably contain 10-20 morphologically and functionally distinct types of each. Because the density of each subtype is relatively low, a micropipette advanced through the retina encounters few examples of any given neuronal type. Moreover, the amacrine cells are quite small (8-14 um), so that only a direct hit with the micropipette is likely to yield successful penetration of the neuron.

We have been studying an amacrine cell, present in the rabbit retina, that synthesizes and releases acetylcholine. These neurons have been identified by autoradiography (Masland and Mills, 1979). Their shape is partly revealed by neurofibrillar staining (Vaney et al., 1981) and has been inferred by comparison of Golgi-stained cells with the distribution of acetylcholine revealed autoradiographically (Famiglietti, 1983). In an attempt to more definitively learn their shape, a method in which they are selectively injected with marker compounds after staining of their nuclei with a fluorescent dye has been developed.

The dye 4,6, diamidino-2-phenylindole (0.5 ug in H_2O) was injected intravitreally. Two days later the retina was removed, fixed for 15 minutes in 4% paraformaldehyde, and mounted flat. For unknown reasons, the blue fluorescent dye is selectively accumulated by the cholinergic amacrine cells (Masland, 1983). In a fluorescence microscope, the use of pipettes filled with lucifer yellow CH or a mixture of lucifer and horseradish peroxidase then allows penetration of the labeled neurons under visual control. Fixation of the retina does not destroy the integrity of the neuronal membranes, so that lucifer injected into a cell fills its processes. Neurons of retinas stored at 4^0 in 0.1M phosphate buffer could be filled successfully 1 month after fixation.

Eighty dye-labeled amacrine cells of the ganglion cell layer were successfully injected, at a variety of retinal locations. They were found to form a single morphological class, with unistratified dendritic arborization in a flat plane. They have very thin dendrites containing, in their distal third, abundant varicosities. A mirror-symmetric population of cells was identied in the inner nuclear layer. These neurons appear identical to the "starburst" amacrines seen in Golgi material, and to the matching populations identified by neurofibrillar staining.

Lucifer injection into fixed cells has been used here exclusively as an anatomical method. Because fluorescent dyes that stain living retinal neurons are available, the method can perhaps be employed for physiological studies as well.

Supported by NIH grant #EY 01075.

262.6 D-ASPARTATE POTENTIATES THE EFFECTS OF BOTH L-ASPARTATE AND L-GLUTAMATE ON HORIZONTAL CELLS IN CARP RETINA. M. Ariel* and S. C. Mangel* (SPON: P. H. Hartline). The Biological Laboratories, Harvard University, Cambridge, MA 02138

It has recently been reported that D-aspartate potentiates the effect of L-glutamate on retinal horizontal cells by 15 times (Ishida and Fain, 1981). It was suggested that D-aspartate exerts this effect by saturating the high affinity uptake mechanisms for acidic amino acids known to be present in the retina. We have further examined the effects of D-aspartate and find that this drug enhances the effectiveness of both L-glutamate and L-aspartate upon carp horizontal cells.

D-aspartate, L-aspartate and L-glutamate were applied to the isolated carp retina by superfusion or by atomization. Upon the addition of D-aspartate (3 mM) to the superfusate, H1 horizontal cells were transiently depolarized by 5-15 mV. Subsequently, the responses of both L-aspartate and L-glutamate were potentiated. Additional applications of D-aspartate led to larger transient depolarizations with longer times required for membrane potential recovery. Application by atomizer of D-aspartate (25 mM in the atomizer) had little or no effect on the membrane potential or light response. However, the effects of both L-aspartate and L-glutamate were again greatly enhanced immediately following D-aspartate application. The increase in effectiveness of both these amino acids was observed in the atomization experiments in L-type and C-type cone horizontal cells, in dark-adapted and light-adapted retinas, and in Co^{++} treated retinas in which synaptic activity was blocked. Furthermore, in these experiments, L-aspartate and L-glutamate effects returned to normal within a few minutes. Since L-aspartate and L-glutamate are taken up by the same high affinity uptake mechanism (Marc and Lam, 1981; Drejer et al., 1983) our results are consistent with the idea that D-aspartate exerts its sensitizing effects principally by blocking amino acid uptake in the retina. The potent uptake system for acidic amino acids in the retina may be responsible for the 25-200 fold difference in L-glutamate sensitivity of horizontal cells in the intact retina, as compared to isolated horizontal cells (Mangel et al., 1983).

262.7 THE EFFECT OF 2-AMINO-5-PHOSPHONOVALERATE ON RESPONSES OF MUDPUPPY RETINAL GANGLION CELLS. P.D. Lukasiewicz* and J.S. McReynolds, Department of Physiology, The University of Michigan, Ann Arbor, MI 48109.

Responses to aspartate, glutamate and related amino acids may be mediated by pharmacologically different receptor sites, which have been classified as quisqualate (QA), kainate (KA) and N-methyl-D-aspartate (NMA) based on the relative sensitivity to these three aspartate/glutamate analogues.

We tested the relative effects of QA, KA and NMA on mudpuppy ganglion cells. All three substances caused depolarization and increased conductance. In all ganglion cells (ON-center, OFF- center and ON-OFF) the order of potency of the analogues was QA $\geq$ KA $>$ NMA. The specific NMA receptor antagonist 2-amino-5-phosphonovalerate (APV) blocked the light-evoked sustained excitatory and inhibitory responses of ganglion cells. Transient inhibitory responses at on and off were never blocked by APV. The transient EPSPs at on and off were greatly reduced in some ON-OFF ganglion cells but not in others. Furthermore, in some ON-center ganglion cells blocking the sustained depolarizing response revealed APV-resistant transient EPSPs at on and off. The responses of bipolar cells were not blocked by APV.

Thus, the sustained light-evoked responses and a subclass of excitatory transient responses in ganglion cells appear to involve NMA receptors. Whether the APV-sensitive receptors are located on amacrine, ganglion or both cell types is not yet known. The APV-resistant excitatory responses in ganglion cells may involve QA or KA receptors or transmitters other than amino acids. Additional work will be necessary to distinguish between several possible wiring schemes which are consistent with these results.

The sensitivity of ganglion cells to NMA, but not to QA or KA, was greatly reduced when transmission was blocked by 4 mM cobalt. This suggests that the NMA receptors, and hence the sites of APV sensitivity, are on cells which are presynaptic to ganglion cells and postsynaptic to bipolar cells, i.e., amacrine cells. However, we have not yet encountered cells of any type which responded well to NMA in the presence of cobalt. Alternatively, it is possible that ganglion cells do have NMA receptors which are directly blocked by a postsynaptic action of cobalt (Ault et al, J. Physiol. 307: 413-428, 1981).

Supported by NIH Research Grant EY 01653.

262.8 RETINAL AND HYPOTHALAMIC DOPAMINE BIOSYNTHESIS IN VITRO: EFFECT OF HYPERPROLACTINEMIA AND HYPOPHYSECTOMY. D.K. Sundberg*, B.A. Bennett* and W.K. O'Steen (SPON: D.J. Goode). Depts. of Physiol. & Pharmacol. and Anatomy, Bowman Gray Sch. Med., Winston-Salem, NC 27103.

Previous reports have demonstrated that prolactin augments light induced photoreceptor damage in the hypophysectomized rat. Prolactin also stimulates tuberoinfundibular dopaminergic (DA) activity. Since the retina possesses an active DA system, one might postulate the existence of a prolactin-amacrine-dopamine photoreceptor interaction. To investigate this hypothesis we examined the effect of 1) anterior pituitary autotransplant induced hyperprolactinemia and 2) hypophysectomy on the in vitro biosynthesis of retinal and hypothalamic DA. Male rats were made hyperprolactinemic by transplantation of pituitaries under each kidney capsule. Hypophysectomized rats were purchased from a commercial vendor. At sacrifice the retinas and medial basal hypothalamus (MBH) were removed and incubated in a balanced salt solution containing 5 μCi of 3H 2-6, tyrosine (30 Ci/mM). After incubation the tissue homogenates were extracted with alumina. DA was separated by reverse phase chromatography and measured by amperometric detection. Newly synthesized DA was determined by scintillation counting. Prolactin levels were increased by autotransplants coincident with an elevation of MBH DA synthesis. However, retinal DA biosynthesis was not affected by this manipulation. Hypophysectomy which alters the entire endocrine system stimulated both retinal and hypothalamic DA biosynthesis (see table).

	Retina (R)		MBH	
	DA ng/R	DA dpm/ng	DA pg/mg	DA dpm/ng
Control	3.9 ± 1.4	181 ± 18	877 ± 65	86 ± 16
Hyperprol.	4.5 ± 0.9	186 ± 22 NS	682 ± 11	179 ± 25*
Control	2.3 ± 0.3	662 ± 43	331 ± 33	97 ± 27
Hypox.	1.8 ± 0.3	946 ± 80*	270 ± 30	217 ± 7*

* = $p < 0.05$

These results suggest that the amacrine DA system is not involved in the augmentation of photoreceptor damage by prolactin.

(Supported by NIH grants HD 10900 to DKS and EY 02359 to WKO)

262.9 DO MAJOR PHYSIOLOGICAL RETINAL GANGLION CELL CLASSES HAVE DISTINCT MORPHOLOGIES? F.R. Amthor, C.W. Oyster and E.S. Takahashi. School of Optometry, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

The rabbit retina has at least six major ganglion cell classes with complex response properties. These include two distinct direction-selective (DS) cell types, orientation selective cells, local edge detectors, large field units, and uniformity detectors. In addition, a number of concentric cell class subdivisions have been described. Anatomical classifications with as many as 23 distinct morphologies (cat retina: Kolb et al., Vis. Res. 21, 1981) implicitly suggest that major morphological differences should have some functional significance. The converse hypothesis is that cell classes with important physiological differences should have distinct morphologies.

To test this hypothesis, we have used intracellular recording and staining. Although our sample is still somewhat small, we have recovered ganglion cells representing many of the major physiological classes. The uniqueness hypothesis, and several other structure/function conjectures remain tenable. Specifically, 1) When the excitatory receptive field center response is On, Off, or On-Off, the corresponding ramification of the ganglion cell dendrites is in the inner IPL, outer IPL, or both, respectively. 2) Two types of On-Off responding cells (local edge detectors and On-Off DS cells) are distinguishable in both flat mount morphological characteristics and in IPL stratification pattern, although both cell types have dendrites in both the inner and outer IPL. Interestingly, although On-Off DS cells are distinguishable as a class from other morphologies, the preferred direction is not obviously apparent from their global dendritic tree structure. On-type DS cells, in contrast, are unistratified and have a less complex flat mount dendritic tree structure. 3) Present data suggest that uniformity detectors have a complex regional multistratification pattern with radially asymmetric locations of dendritic specializations.

Although the concentric cells recovered so far appear to be distinguishable as a group from the complex types, insufficient data has been obtained to argue for a unique morphology for each subclass. It is apparent, however, that at least three distinguishable morphologies underlie different concentric cell types. In contrast with other areas of the visual system, optical geometrical constraints and local circuit interactions may require that specific ganglion response properties be dependent on particular morphological structures.

Supported by EY02207, EY03895, EY03039 (CORE), and RR05807 (BRSG).

262.10 CO-LOCALIZATION OF (^{3}H)-GLYCINE UPTAKE AND NEUROTENSIN-LIKE IMMUNOREACTIVITY IN SUSTAINED AMACRINE CELLS OF THE TURTLE RETINA. R. Weiler*, A.K. Ball* and W.K. Stell. Dept. of Anatomy and Lions' Sight Centre, University of Calgary, Calgary, Alberta, Canada. T2N 4N1

The eye of the turtle, Pseudemys scripta, was injected with (^{3}H)-GABA and (^{3}H)-glycine in order to identify amacrine cells which have a high affinity uptake system for these amino acids, and may use them as neurotransmitters. These retinas were then treated with antibodies against different neuropeptides using the Peroxidase-Antiperoxidase (PAP) technique to reveal immunoreactivity prior to autoradiographic localization of (^{3}H)-GABA and (^{3}H)-glycine.

(^{3}H)-glycine is accumulated by many cells whose somata are located in the amacrine cell layer. A small proportion of these cells also are neurotensin-immunoreactive. These doubly labeled cells have a small cell body and fine processes that spread diffusely throughout the inner plexiform layer (IPL). Neurotensin-immunoreactive cells of a second type, which do not accumulate (^{3}H)-glycine, have large cell bodies and one major neurite that projects to the mid-IPL where it forms a monostratified arborization. Neurotensin-immunoreactive cells that take up (^{3}H)-GABA were not observed.

In parallel experiments we attempted to learn the function of these cells by means of intracellular recording followed by injection of Procion Yellow or horseradish peroxidase. The marker-injected cells that most closely resemble the cells doubly labeled by neurotensin-immunoreactivity and (^{3}H)-glycine uptake respond to light with a sustained depolarization of their membrane potential.

Supported by Alberta Heritage Foundation for Medical Research, Medical Research Council of Canada, and Schweizerische Stiftung für Medizinische-Biologische Stipendien.

262.11 ^{3}H-GABA RELEASE FROM MÜLLER (GLIAL) CELLS IN THE RAT RETINA. P. V. Sarthy. Dept. of Ophthalmology, Univ. of Wash., Seattle, WA 98195.

In several neural systems, glial cells appear to take up and release GABA upon depolarization. We have studied the release of ^{3}H-GABA from Müller (glial) cells in the rat retina by a double isotope-labeling procedure in which Müller cells are preloaded with ^{3}H-GABA while a population of neurons are prelabeled with ^{14}C-glycine. By autoradiography, we have confirmed that ^{3}H-GABA is taken up by the radially-oriented Müller cells while ^{3}H-glycine is accumulated by a subset of amacrine cells (neurons) in the rat retina. Using the double-labeling procedure, we have examined the effects of two depolarizing agents, high [K^+] and veratridine, and the GABA mimetic, ethylenediamine, on transmitter release from glial cells and neurons simultaneously. We found that (1) depolarization with 56 mM [K^+] released both ^{3}H-GABA and ^{14}C-glycine. About 70-80% of this release was blocked in Ca^{2+}-free medium; (2) 10 µM veratridine also released both the transmitters. This release was inhibited by 100 mM Tetradotoxin or 1 mM Procaine. Under Ca^{2+}-free conditions, < 20% isotope release was observed; (3) ethylenediamine (EDA) released ^{3}H-GABA readily while little ^{14}C-glycine release was observed. Removal of Ca^{2+} had no significant effect on the release of either transmitter. Further, in Na^+-free medium EDA failed to induce ^{3}H-GABA or ^{14}C-glycine release. These results suggest that high [K^+] and veratridine release ^{3}H-GABA from Müller cells by a Ca^{2+}-dependent vesicular process. Ethylenediamine, on the other hand, appears to induce ^{3}H-GABA release by a Ca^{2+}-independent, carrier-mediated exchange mechanism.

Supported by NIH Grants EY-03523, EY-03664 and EY-01730.

263.1 A NEURITE-PROMOTING ACTIVITY PRESENT IN HEART CELL CM ENHANCES THE NGF-INDUCED CONVERSION OF CHROMAFFIN CELLS TO NEURONS. A.J. Doupe and P.H. Patterson, Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

A variety of conditioned media (CM) contain factors which bind to substrates and markedly enhance neurite outgrowth of peripheral neurons in vitro. One such activity, from PC12 cell CM, is associated with a heparan-sulfate proteoglycan fraction (HeS)(Matthew et al., Soc. Neurosci. Abst. 8:83.4, 1982). Initial neurite outgrowth of sympathetic neurons on this HeS is independent of nerve growth factor (NGF), although NGF is required for long-term survival of these cells in vitro.

Adrenal chromaffin cells, like sympathetic neurons, are neural crest-derived, but do not have neurites and do not require NGF for survival. However, ~20% of newborn rat adrenal chromaffin cells respond to NGF administration in vitro by growing neurites and eventually becoming indistinguishable from mature sympathetic neurons (Doupe et al., Soc. Neurosci. Abst. 8:70,5, 1982). When we culture newborn chromaffin cells in the presence of NGF on a surface coated with serum-free rat heart cell CM, the percentage of cells that become neurons is greatly increased, as is the rate of conversion. Thus CM contains a factor which enhances the NGF-induced transformation. Administration of CM in the absence of added NGF also elicits a small amount of process outgrowth from chromaffin cells, although the cells do not progress to the completely differentiated neuronal phenotype. The conversion-enhancing activity appears to be similar to the neurite-promoting activity from PC12 cell CM. A monoclonal antibody (4H8) to the latter preparation, known to block directly the activity of PC12 cell CM on neurons (Matthew & Patterson, this vol.), also completely blocks the enhancement of chromaffin cell conversion to neurons when adsorbed onto the heart CM-treated surface.

Unlike cells from newborn medullae, few adult rat chromaffin cells grow processes in response to NGF. However, addition of heart cell CM markedly increases the number of cells that respond. Corticosteroid normally blocks the effect of NGF on chromaffin cells (Unsicker et al., PNAS 75:3498, 1978). In the presence of CM, on the other hand, dexamethasone is completely ineffective in blocking the neurite outgrowth. Our results suggest that, in addition to soluble environmental signals such as NGF and corticosteroids, extracellular matrix molecules like the HeS-associated activity described here also have important effects on developmental decisions in the sympatho-adrenal lineage.

Supported by the McKnight & Rita Allen Foundations, the NINCDS, and the Harvard Univ. Soc. of Fellows.

263.2 EXPRESSION OF SOMATOSTATIN IMMUNOREACTIVITY IN NEURAL CREST CULTURES G.D. Maxwell, P.D. Sietz*, and S. Jean* Dept. of Anatomy, Univ. of Connecticut Health Center, Farmington, CT 06032

We are studying the development of neuronal properties by neural crest cells in tissue culture. Previous work has shown that some cells in neural crest cultures acquire the capacity to synthesize and store catecholamines and to take up [^{3}H]norepinephrine and release it in response to depolarization. We report here that a subpopulation of cells in neural crest cultures develop the capacity to express somatostatin-like immunoreactivity (SLI) after several days in vitro. Some cells in these cultures contain both catecholamines and SLI.

Neural crest cultures from the trunk region of quail embryos were prepared as described previously (Maxwell et al., 1982 J. Neurosci. 2:879-888). Growth medium contained 15% horse serum, 10% chick embryo extract, and 5 ng/ml 2.5S NGF. Using immunocytochemistry, cells with SLI are not detected prior to 5 days in vitro. At 5 days in vitro a few cells with faint immunoreactivity are observed. By 6 days in vitro the number of SLI positive cells has increased by an order of magnitude and the intensity of the immunoreactivity has increased markedly. At 9 days in vitro SLI positive cells constituted about 0.1% of the total cell population. The number of cells with SLI increased approximately linearly until 10 days in vitro (the latest time examined). The immunoreactivity is present in the cytoplasm and absent from the nucleus. SLI containing cells with and without processes are observed. The SLI positive cells are often clustered near one another.

Experiments which first detected catecholamine-containing cells histochemically followed by immunocytochemistry for SLI showed that some cells which contain catecholamines also contain SLI. Cells containing both traits were observed as early as 7 days in vitro and as late as 14 days in vitro (the earliest and latest times examined). On the average about 20% of catecholamine-positive cells were also positive for SLI. In these cultures treated to reveal catecholamines and SLI, the number of SLI positive cells was comparable to that seen in cultures processed for SLI alone.

These findings demonstrate that the expression of SLI by some neural crest cells can occur in vitro in the absence of their normal cellular environment. In addition, in some cases the same cells contain both catecholamines and SLI.

(Supported by Basil O'Connor Starter Grant 5-289 from the March of Dimes Birth Defects Foundation and grant NS16115 and a Research Career Development Award (GDM) from the NIH).

263.3 ADRENERGIC DIFFERENTIATION OF NEURAL CREST CELLS MAY BE DEPENDENT UPON A NEURAL TUBE DERIVED FACTOR. Marthe J. Howard* and M. Bronner-Fraser (SPON: M. Cahalan). Department of Physiology and Biophysics, University of California, Irvine, CA 92717.

Neural crest cells give rise to numerous derivatives including melanocytes, adrenergic and cholinergic neurons, and supportive cells of the nervous system. We have previously shown (Howard, et al. Soc. Neurosci. 8(1): 257, 1982) that cultured neural crest cells will differentiate into adrenergic neuroblasts or pigment cells when grown in medium containing 10% embryo extract. This effect was demonstrated for neural crest cells grown with or without the presence of the neural tube. However, when the embryo extract concentration is decreased to 2%, no catecholamine production (assayed by formaldehyde-induced-fluorescence) was observed. In the present study, we examine whether a factor or factors elaborated by the neural tube will support adrenergic differentiation.

Conditioned medium from neural tube, somite, or notochord cells was obtained by growing the respective cell type in culture in medium supplemented with horse serum and 2% embryo extract. The conditioned medium was harvested from these embryonic tissues every other day. Neural crest cells were subsequently fed the conditioned medium. When neural crest cells were grown in medium containing 2% embryo extract which was previously conditioned by neural tube, adrenergic neuroblasts were observed in all cultures. This effect appears to be at least partially specific since somite and notochord conditioned medium did not support adrenergic expression. Pigmented cells were present in all cultures, with or without neuronal differentiation, indicating the general health of the cells. To test the possible role of direct contact with the neural tube, neural crest cells were plated onto a layer of neural tube membranes in the presence of 2% embryo extract. No adrenergic differentiation was observed under these conditions.

The results indicate that: 1) the neural tube makes a factor or factors that will support adrenergic differentiation; and 2) contact with the neural tube is not required for expression of the phenotype.

(Supported by USPHS Grant HD-15527-01 and March of Dimes Grant D-312)

263.4 IN VITRO DIFFERENTIATION OF QUAIL NEURAL CREST CELLS INTO SEROTONINERGIC, MET-ENKEPHALIN-IMMUNOREACTIVE AND SOMATOSTATIN-IMMUNOREACTIVE NEURONS. Maya Sieber-Blum. Department of Cell Biology and Anatomy, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

A wide variety of vertebrate cell types and tissues are the progeny of the same transient embryonic structure, the neural crest. They include much of the peripheral nervous system, nerve-supporting cells, pigment cells, endocrine cells, and head mesenchyme. Methods for the in vitro culture of neural crest cells holds promise for a detailed analysis of some aspects of neural crest cell development under well controlled conditions and insights into the underlying cellular and molecular regulatory mechanisms. However, thus far, only a few differentiated phenotypes have been identified among cultured neural crest cells: adrenergic neurons, cholinergic neurons, melanocytes and chondrocytes. In this communication, we report the in vitro differentiation of three additional neural crest-derived cell types: serotoninergic, met-enkephalin-immunoreactive and somatostatin-immunoreactive neurons. Serotoninergic neurons were identified by formaldehyde-induced histofluorescence and by indirect staining with an anti-serotonin antibody in both the presence and absence of the monoamine oxidase inhibitor, Parnate. No enhancing methods were used to identify peptidergic neurons by indirect immunofluorescence. The antibodies stained perikaryon and cell processes in all three cell types. Serotoninergic neurons were uni- or multipolar with short cell processes. Somatostatin-immunoreactive neurons were multipolar and had extremely fine and long cell processes with numerous varicosities and elaborate ramifications. By contrast, met-enkephalin-immunoreactive cells had few, very short and rather coarse processes. Contrary to expectations, serotoninergic neurons were found in neural crest cultures derived from all levels of the neuraxis, including the thoracic/upper lumbar level. In vivo crest cells from the thoracic/upper lumbar axial levels give rise to the sympathetic system which is predomonantly adrenergic. They do not contribute to the enteric ganglia which contain serotoninergic neurons. The results presented in this communication may indicate that the sympathetic nervous system of the quail contains serotoninergic cells. Alternatively, it is also conceivable that some restriction mechanisms that are active in vivo are abolished in culture and thus allow neural crest cells from certain axial levels to express a larger segment of their developmental potential. (Supported by NIH Grant HD 15311)

263.5 DIFFERENTIATION AND TRANSFORMATION OF NEURAL PLATE CELLS. R.W. Keane*, L.A. Lipsich*, and J.S. Brugge* (SPON: E.F. Barrett). Dept. of Physiol. Biophys. Univ. Miami School of Med., Miami, FL 33101, and Dept. of Microbiology Basic Health Sciences, SUNY at Stony Brook, Stony Brook, NY 11794

The developmental potential of presumptive neural plate cells of pre-streak chick embryos (stage 1) and primary-induced neural plate cells from definitive streak chick embryos (stage 4) has been examined in cell culture using specific markers which identify the major cell types in the vertebrate central nervous system. The pre-streak presumptive neural plate (PSPNP) cells, stage 1, which have not been determined, assume an epithelial appearance in vitro and synthesize cellular fibronectin, but do not express markers for the neuronal, astrocytic, melanocytic or oligodendrocytic lineages. Conversely, primary-induced definitive-streak neural plate (DSNP) cells are competent to express cell-type-specific markers for terminally differentiated neurons, astrocytes, and melanocytes, and synthesize an extracellular matrix of cellular fibronectin. Differentiation of DSNP cells in vitro can be prevented by infection with a temperature-sensitive mutant of Rous sarcoma virus, tsNY68. The development of DSNP cell transformants can be resumed by a temperature shift to the nonpermissive temperature. The morphological and biochemical changes associated with tsNY68 transformation are accompanied by alterations in $pp60^{src}$ kinase activity in the transformed cells.

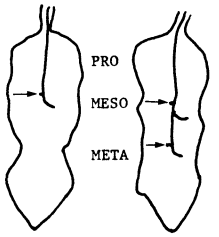
263.6 DUPLICATION OF NEURAL STRUCTURE IN BITHORAX FLIES. John B. Thomas* and Robert J. Wyman. Department of Biology, Yale University, New Haven, CT 06511 USA.

Homeotic mutations change the state of determination of one body part to that of a different body part. In Drosophila certain mutations within the bithorax gene complex (BX-C) transform metathorax into mesothorax. Although the cuticular transformations are well described, the effect of these mutations on internal tissues is less certain. This is due in large part to the lack of suitable internal markers for segmental identity.

The branching pattern of the Drosophila giant fiber neuron (GF) offers an unambiguous marker for the mesothoracic segment of the nervous system. The GF cell body lies in the brain; its axon descends into the thoracic ganglion. The axon has no branches in the prothorax and does not enter the metathorax (Brain Research 221:213). Within the mesothoracic neuromere it extends a tuft of small processes (arrows) which synapse onto the peripherally synapsing interneuron and it also bends laterally to synapse onto the tergotrochanteral motorneuron (J. Neurocytol. 9:753).

Mutant bithorax flies of the genotype abx bx^3 pbx/Df(3R)P2 (Drosophila Inf. Serv. 55:207) show nearly complete meta to mesothoracic cuticular transformation. In these flies the GF mesothoracic branches (revealed by intracellular injection of Lucifer Yellow) are duplicated in the metathoracic neuromere. Thus the normal mesothoracic cues involved in GF branching appear to be duplicated in the metathoracic neuromere of the BX-C mutant flies. These changes could be due to a direct affect of the BX-C mutations on the CNS or could be due to an inductive effect from other homeotically transformed tissues such as the cuticle or sense cells.

Giant Fiber Neuron in Thoracic Ganglion



263.7 REORGANIZATION OF SEGMENTAL FOUNDER CELLS IN THE LEECH EMBRYO FOLLOWING MICROBEAM ABLATION OF CELLS LABELLED WITH A FLUORESCENT LINEAGE TRACER. M. Shankland. Dept. of Molecular Biology, U. of California, Berkeley, CA 94720.

The egg of the leech Helobdella triserialis develops directly into the adult form through a stereotyped sequence of cell divisions, and it is possible to identify specific segmental founder cells, called blast cells, on the basis of their lineage. These blast cells lie in 5 parallel germinal bandlets, with each bandlet arising from a larger teloblast by a series of asymmetric divisions. Injecting teloblasts with cell lineage tracers shows that each bandlet gives rise to a distinctive set of segmentally iterated tissues, and that the blast cells within a bandlet are ancestral to segmentally homologous tissue complements. However, the blast cells within a given bandlet must give rise to slightly different descendant clones in order to account for the known differences between the individual segments.

Small groups of blast cells were ablated by means of a photosensitizing lineage tracer, fluorescein dextran (FDX), injected into and inherited from the parental teloblast. FDX does not interfere with development under normal conditions, but sensitizes cells to light within the fluorescein absorption spectrum. FDX-labelled blast cells were lesioned under an epi-fluorescence microscope equipped with a 50 W Hg arc light source and a narrow band exciter filter with peak transmittance at 480 nm. Using a 40X objective, a 20-60 sec exposure causes labelled blast cells to undergo rapid degeneration resulting within 2-10 hr in a loss of membrane integrity and dissipation of the tracer. This microbeam could be narrowed so as to selectively lesion 2 adjacent blast cells, thus sundering the labelled bandlet into separate rostral and caudal fragments. Unlabelled blast cells have negligible absorption at these wavelengths, and can be illuminated for up to 300 sec without any apparent damage.

There is normally a strict correlation between a blast cell's birth rank and its segmental fate. However, if a bandlet is photolesioned at the point where it merges with the other bandlets, the fragment caudal to the lesion is temporarily retarded and consequently slips out of its normal segmental register. The slipped bandlet is frameshifted so that its blast cells come to occupy segments caudal to those for which they are normally destined, where they produce their normal bandlet-specific complement of tissues. This segmental reorganization allows one to ask whether certain segment-specific features of the blast cell clones are determined by blast cell birth rank or by the position of the descendant clone along the rostrocaudal axis. The results show that at least one such segmental difference--the death of the supernumerary blast cells--is determined by position irrespective of birth rank.

263.8 DEVELOPMENTAL INDETERMINACY IN THE LEECH EMBRYO. D.A. Weisblat and M. Shankland. Molecular Biology, Univ. of Calif., Berkeley, Berkeley, CA 94720.

Identifiable neurons in the segmental nervous system of the leech derive from bilateral pairs of blastomeres, the left and right M,N,O,P and Q teloblasts. The teloblasts themselves are identifiable by size, position, or (except for O and P) by the order in which they arise during cleavage. The teloblasts make smaller blast cells in columns called germinal bandlets; these coalesce in a fixed manner ipsilaterally into left and right germinal bands, then rostrocaudally along the ventral midline into a structure called the germinal plate, from which the segmental tissues, including the ganglia of the ventral nerve cord, arise. Labeling individual teloblasts with cell lineage tracers revealed that each blast cell normally generates a distinct segmental complement of progeny, including neurons, which is characteristic of its teloblast of origin. This would suggest that neuronal fates might be determined at the time of teloblast formation, but in fact two lines of evidence have indicated that the O and P teloblasts (equivalent in their lines of descent from the egg) are also of equal developmental pluripotency, with the fates of their neuronal progeny being determined by a hierarchical interaction between blast cells in the germinal band (NS abst. 70.2, 1982). Firstly, in about one third of the embryos, positionally identified O teloblasts give rise to P teloblast progeny distribution patterns and vice versa; in fact it is the relative positions of the o and p germinal bandlets within the germinal band, not the position of the teloblasts, which correlates with the progeny distribution pattern. Secondly, in experiments where either an O or a P teloblast was ablated by DNase injection, the surviving ipsilateral P or O teloblast's progeny generated the P pattern of neurons, with the usual O progeny being absent.

Results to be presented, from experiments using the photoablation technique described in the previous abstract, confirm and extend the previous work as follows: firstly, when the posterior part of a bandlet whose anterior blast cells are already making the P pattern is caused to grow between the n and o bandlets of the contralateral germinal band, these posterior blast cells give rise to the O pattern of neurons, showing directly that P- as well as O-derived blast cells can make either the O or the P pattern; secondly, by lesioning p blast cells at various times after they have already entered the germinal band, we have found that the o blast cells become committed to making the O pattern at or near the time of their first mitosis.

263.9 REGULATION AND DETERMINATION OF NEURONAL PRECURSOR CELLS REVEALED BY CELL ABLATIONS IN THE GRASSHOPPER EMBRYO. Chris Q. Doe* and Corey S. Goodman (SPON: S. Glotzbach). Dept. of Biol. Sci., Stanford University, Stanford, CA 94305.

Each segmental ganglion of the grasshopper CNS arises during embryogenesis from a stereotyped pattern of neuronal precursor cells in the neuroepithelium: 61 neuroblasts (NBs) and 7 midline precursors. The NBs are arranged in two plates of 30 NBs, each composed of 7 NB rows, and 1 median NB. NBs are stem cells which divide repeatedly to produce families of neuronal progeny. Each NB can be individually identified according to its position and the highly stereotyped family of identified neurons it produces. We are interested in how the ectoderm gives rise to the NBs, and how the NBs acquire their unique identities.

Immediately following gastrulation the embryo is a thin strip of ventral ectoderm and dorsolateral mesoderm. The middle portion of the ectoderm becomes the neuroepithelium as cells delaminate and enlarge to form NBs. All 30 NBs within the plate do not form simultaneously. Rather, as the embryo grows, gaps develop between the initial NBs as additional ectodermal cells delaminate to form small clusters of 2-8 cells between the older NBs. Ultimately, only one cell within the cluster enlarges and becomes a NB; the remaining cells either die or form ensheathing cells around the NB and its progeny. In the 7th row, NBs 7-1 and 7-4 form first, followed by NB 7-2, and lastly NB 7-3. Before NB 7-3 appears, a cluster of 2-8 cells are situated between NB 7-2 and NB 7-4; one of these cells enlarges to become NB 7-3.

We have used either a laser microbeam or a microelectrode to ablate individual cells or specific groups of cells during the development of NBs 7-2 and 7-3. When we ablate the entire cluster of 2-8 small cells normally destined to form one of these NBs, no NB appears. When we ablate only a portion of the cluster, however, the NB forms. In particular, when we kill only the cell that is enlarging to form the NB, one of the remaining cells becomes the NB. It appears as if the NB can form from any one of the cells within the cluster, even after one cell has begun enlarging. We conclude that cell interactions within the cluster determine which cell becomes the NB, and that regulation can occur early in this process. However, once the enlarged NB begins its first division, no regulation occurs; ablation leads to a lack of the NB, despite the existence of delaminated cells adjacent to the ablated NB. Interestingly, when the mature NB 7-4 is ablated at a time when the cluster of cells for NB 7-3 is adjacent, NB 7-3 can appear but NB 7-4 is never replaced.

We conclude from these results that any cell within a cluster has the potential to make the correct NB (for that position within the neuroepithelium), but lacks the potential to make the adjacent NB. This suggests that particular clusters of delaminated ectodermal cells may represent precursor populations for a particular NB. Furthermore, by the time of its first division, the NB cannot be replaced; the remaining cells within the cluster, and the overlying epithelial cells, have either become commited to other fates, or are no longer capable of acquiring the NB fate. (Supported by a NSF predoctoral fellowship to CQD and a NIH grant to CSG.)

263.10 NEURONAL DETERMINATION DURING EMBRYONIC DEVELOPMENT: HIERARCHICAL FATE OF TWO SIBLING CELLS IN THE GRASSHOPPER CNS. J.Y. Kuwada and C.S. Goodman. Dept. Biol. Sci., Stanford Univ., Stanford, CA

We are studying the roles of cell lineage and interactions in the determination of identifiable neurons in the CNS of the grasshopper embryo. Two morphologically distinct sibling neurons, the H cell and the H cell sib, are the progeny from the symmetric division of midline precursor 3 (Goodman, Bate, & Spitzer, J. Neurosci., 1981). The two MP3 progeny are well suited for this study since they are easily identified by their unique location, can be filled intracellularly with dye, and can be easily manipulated. Here we describe the results following ablation of one of the two progeny at various stages after their birth. Cells were individually ablated with a microelectrode and the embryos allowed to develop in culture.

When the precursor cell divides, two progeny of equal size are produced which are located just ventral to the dorsal basement membrane. Ten hours later the first signs of differentiation appear: growth cones extend and one progeny moves away from the basement membrane making it ventral to its sibling. Thirty six hours following mitosis the two progeny are clearly distinguishable with different patterns of axons: the dorsal progeny is the H cell while the ventral progeny is the H cell sib. This pattern of development is reproduced in control ganglia when embryos are allowed to develop in a culture system.

When one of the progeny was ablated within 5 hours of the precursor mitosis and allowed to develop in culture for sufficient time, only one progeny was present and it possessed the H cell sib morphology in 11 of 14 cases and the H cell morphology in the remaining 3 cases. Thus at this time the embryo cannot number regulate, i.e., compensate for the loss of one progeny to restore the original number of progeny. Moreover, the progeny are not yet individually determined for the most part. Instead the surviving progeny appears to regulate its fate in response to the ablation of its sibling. However, when one progeny is ablated 10-15 hours following mitosis, the remaining progeny can be either the H cell or the H cell sib with equal facility. If the dorsal progeny was ablated, the survivor was the H cell sib (n=6); if the ventral progeny was ablated, the survivor was the H cell (n=2); and when one progeny was ablated and the dorsal-ventral relationship of the two progeny was not clear (n=10), 50% of the survivors were H cells and 50% H cell sibs. Thus as little as 5 hours later the progeny are determined and act autonomously in response to ablation of their sibling.

We conclude that within 5 hours of precursor mitosis, fate regulation can occur in response to ablation of one of the sibling progeny. This suggests that a hierarchy of cell fates governed by interactions with other cells or non-cellular elements exists for these sibling neurons. Additionally, the interaction occurs in a specified short period of time prior to the first signs of differentiation, number regulation does not occur, and the two progeny are not dependent on each other for survival. Presently neighboring neuronal progeny are being ablated in hopes of identifying possible cellular interactions. (Supported by NIH fellowship to JYK and an NIH grant to CSG.)

263.11 NEUROTRANSMITTER DETERMINATION: EMBRYONIC AND POSTEMBRYONIC DEVELOPMENT OF THE NEUROPEPTIDE PROCTOLIN IN THE GRASSHOPPER CNS. H. Keshishian and M. O'Shea. Committee on Neurobiology, The University of Chicago, Chicago, Il. 60637.

The development of the neuronal expression of proctolin was studied in embryos and nymphs of the grasshopper S. nitens, using immunohistochemistry, dye-fills, HPLC, bioassay and the incorporation of ^{3}H amino acids. About 45 postembryonic proctolin-IR neurons are present in the ventral nerve cord; somatic and neuropilar staining is stereotyped. The IR cell bodies appear either as clusters or as isolated individuals, a pattern which may reflect different modes of transmitter determination among clonally related neurons. The pro- and mesothoracic ganglia share a pattern of homologous transganglionic arborizations and a pair of ventral midline neurons. In the mesothorax there are also two ventral lateral pairs near the 1st and 2nd nerve roots. The metathoracic ganglion has two pairs of ventral midline interneurons, a pair of ventral neurons in the A3 neuromeres and 5-6 ventral lateral cells among the motorneurons projecting to nerves 3 and 5. On the dorsal posterior side two pairs of cells stain in the nerve 4 motor pool. The darker staining pair may be homologous to the proctolinergic Ds motorneuron of Periplaneta (O'Shea and Bishop, '79, J. Neurosci. 2, 1242). In the terminal ganglion there are two ventral midline clusters of 6-7 neurons each and either 1 large or 2 medium sized Dorsal Unpaired Median (DUM) neurons occuring with equal frequency. Embryonic staining starts at the 60% stage in the brain, with the mature pattern complete throughout the CNS at the 70% stage. The only staining evidence for supernumerary cells was the transient appearance at 70% of a pair of ventral metathoracic A2 neurons. Using C-18 purification levels of 15 femtomoles proctolin/embryo were assayed at the 50% stage. The levels increase with two plateaus; the 1st at the 70% stage after a 20-fold increment, and the 2nd at the 80-85% stage after a 5-fold rise. Peak levels are at the 90% stage, with a 3-fold decline by 100%. ^{3}H amino acid labelling of cultured embryos with HPLC resulted in the incorporation into proctolin during the 1st increment. The 2-stage increase of embryonic proctolin levels is synchronous with two ecdysone pulses and the 2nd and 3rd bouts of cuticulogenesis (Lagueux et al., '79, J. Insect Physiol. 25, 709).

Thus proctolin is a late arising embryonic phenotype that is expressed suddenly in a fixed population of identified post-mitotic cells.

Supported by NIH grant 1 F32 NS06864-01 to HK and by NSF grant BNS-8202515 to MO.

263.12 **A MONOCLONAL ANTIBODY WHICH RECOGNIZES RADIAL GLIAL CELLS IN THE DEVELOPING VERTEBRATE CENTRAL NERVOUS SYSTEM.** **R. McKay and S. Hockfield, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724**

The nervous system is derived from a plate of ectodermal cells which roll up in development to form a tube of approximately 10^{5} cells which proliferate and differentiate to generate the 10^{12} cells and many cell types of the adult vertebrate brain. The application of hybridoma technology to the analysis of the adult vertebrate nervous system has yielded many antibodies which distinguish discrete neuronal types in different areas of the central nervous system, but most of these antibodies failed to bind to the developing neurons. In order to define the major cellular features of the developing brain we have raised monoclonal antibodies to fixed embryonic day 15 spinal cord of rats. At this age the nervous system still contains both the rapidly profilerating epithelial cells and the recently differentiated neurons which have just begun to send out processes and receive input, where cellular differentiation of the neural plate and the initial events in axon outgrowth and synaptic recognition between neurons might be studied. Here we describe an antibody that recognizes a subset of cells in the developing CNS.

The antibody Rat 6-7G12 binds to an antigen which is expressed in radial cells in the columnar epithelium. These cells have the morphological characteristics of the neuroblast cells first described by the Golgi technique and called epithelial cells by Cajal. Immunoblots show the antigen to be a doublet of bands of approximately 180,000 daltons. Both biochemical and histochemical assays show the antigen to be absent in the adult rat brain. At the electron microscopic level the antibody recognizes an internal antigen in a subset of the E15 radial cells, some of these cells contain mitotic figures so we know they are dividing. Other cells in the embryo which express a related antigen are located in the region of the developing somites. Electron microscopy shows the antigen is present in the newly fused myotubes.

The antigens recognized by 7G12 are first apparent in the neural epithelium at the eleventh day of embryonic gestation (E11) and at this stage the majority of radial cells may bind the antibody. At day E15 however the antigen is only present in a subset of radial cells. As development progresses the number of 7G12-labelled cells diminishes, with very few remaining at birth. Antisera against the glial fibrillary acidic protein and vimentin also bind to radial cells in the developing spinal cord. However the antigens recognized by 7G12 have different molecular weights from vimentin and GFAP. Differential expression in early neuronal development of antigens such as these, will allow the analysis of events in neuronal differentiation.

263.13 SELECTION OF CELL-TYPE SPECIFIC MONOCLONAL ANTIBODIES INTERACTING WITH NASCENT POLYPEPTIDE CHAINS SYNTHESIZED *IN VITRO* BY CEREBELLAR mRNA. A. Safran*, Z. Eshhar* , D.M. Phillips* and H. Soreq. Dept. of Neurobiology, Weizmann Institute of Science, Rehovot, Israel.

Utilizing *in vitro* translation of cerebellar mRNA, we have previously found that the production of a number of abundant cerebellar polypeptides is regulated differently during ontogenesis of the cerebellum of normal rats (1), as compared to the irradiation-agranulated rat cerebellum (2) and to the cerebellum of *staggerer* mutants (3). Monoclonal antibodies (mAb) to such polypeptides should enable the assignment of the corresponding proteins to defined cell types and periods in cerebellar development. In order to study the regulation of synthesis of these proteins at the level of mRNA as well, the antibodies should recognize the polypeptide backbone of the proteins. To select for such antibodies, we immunized mice with cerebellar proteins eluted from regions in polyacrylamide gels, where differences in synthesis were observed. Hybridoma cell lines secreting mAb were obtained by fusion with NSO myeloma cells. The antibodies were screened against ^{125}I-labeled cerebellar proteins, that represent the total proteins in the cerebellum, including the contribution of incoming fibers. The mAb were also screened for their binding of ^{35}S-methionine labeled polypeptides, translated *in vitro* from cerebellar mRNA. These represent the biosynthetic potential of endogenous cerebellar cell bodies alone. mAb which were found to be positive by both selection methods were examined for their binding to frozen cerebellar sections by an immunofluorescence technique. In addition to mAb which stained most cell types, we identified mAb which selectively stained specific subcellular regions in various cell types. These include Bergmann fibers of Golgi type II epithelial cells, cell bodies of neurons in both the molecular and the granular layer, and meningeal cells. Such characterized mAb will serve as probes to follow the developmental regulation of the corresponding cerebellar antigenic markers, both at the protein and the mRNA level.

1. Soreq, Safran and Zisling (1982) Dev.Br.Res. 3, 65-79.
2. Eliyahu and Soreq (1982) J.Neurochem. 38, 313-321.
3. Soreq, Safran and Eliyahu (1983) Dev.Br.Res., in press.

263.14 COMPOSITION OF THE PROLIFERATIVE SUBGRANULAR ZONE OF THE DEVELOPING AND MATURE DENTATE GYRUS IN THE RHESUS MONKEY: EM-IMMUNOCYTOCHEMICAL (anti-GFAP) AND AUTORADIOGRAPHIC (^{3}H-TdR) ANALYSES. M. F. Eckenhoff and P. Rakic. Sec. Neuroanat., Yale University School of Medicine, New Haven, CT 06510.

Previous studies using ^{3}H-thymidine (^{3}H-TdR) autoradiography revealed late labeled immature cells in the subgranular zone (SGZ) of the primate dentate gyrus, indicative of a protracted postnatal genesis (Rakic and Nowakowski,'81). A recent study using antiserum to glial fibrillary acidic protein (GFAP) as a glia-specific marker showed the presence of both glial and neuronal cells in the SGZ during prenatal and early postnatal periods (Eckenhoff and Rakic,'83). However, the kinetics of proliferation, the time-dependent changes in the proportions of glial and neuronal cell lines, and the nature of late generated cells needed to be determined. In the present study we employed immunocytochemical localization of GFAP at the EM level and ^{3}H-TdR autoradiography to examine the cytology and proliferative capacity of the SGZ in developing and mature rhesus monkeys.

Vibratome sections of the fixed hippocampus from monkeys at embryonic day 144 (E144), 3, 7, 18 postnatal months and an adult were reacted with GFAP antiserum by the Vectastain ABC procedure and processed for EM analysis. Animals of postnatal ages were injected with ^{3}H-TdR at least two months prior to sacrifice and sections are being processed by combined immunocytochemical and autoradiographic methods. In addition, 5 pairs of monkeys at ages comparable to the above were injected with ^{3}H-TdR and sacrificed either the same day to determine the site of cell genesis, or 2 months later to determine their nature. At E144, the SGZ is 3-4 cells wide and consists of immature GFAP-positive and GFAP-negative cells. These cells are presumably glial and neuronal precursors respectively, since ^{3}H-TdR labeling demonstrates that both cell lineages are generated in the SGZ at this age. At 3 and 7 months, the SGZ becomes 2-3 cells wide and contains a high percentage of GFAP-positive cells. Labeling by ^{3}H-TdR testifies to their proliferative capacity. In the 18 month specimen, the SGZ is reduced to 2 rows of immature cells virtually all GFAP-positive. In the adult monkey the SGZ remains 1-2 cells thick, and most, if not all, are GFAP-positive. So far, our material reveals that: (1) a vestige of the fetal SGZ persists in mature monkeys; (2) initially, the SGZ contains proliferative cells of both glial and neuronal lineages; (3) the number of neuronal cells declines with time such that in the adult virtually all immature and presumably still dividing cells are of astrocytic lineage; (4) in contrast to rodents, where neurons were reported to be added in substantial number to the adult dentate gyrus, in primates the late generated cells from the SGZ appear to be glia. Supported by NS14841.

FEEDING AND DRINKING: CUES FOR NEED STATE III

264.1 REGULATION OF PROTEIN/CARBOHYDRATE RATIO IN FOODS THAT RATS CHOOSE TO EAT. C.L. Thaell*, J.J. Wurtman*, and R.J. Wurtman. Laboratory of Neuroendocrine Regulation, M.I.T., Cambridge, MA 02139.

Ashley and Anderson (J. Nutr., 1975, 105, 1412) have presented evidence that protein intake is regulated separately from energy intake and is inversely correlated with plasma tryptophan/large neutral amino acid ratios. Fernstrom and Wurtman (Science, 1972, 178, 414) have shown that a carbohydrate meal increases the plasma trp/LNAA ratio, increasing brain tryptophan levels and serotonin synthesis, while a high-protein meal has the opposite effect. We have examined the possibility that the regulated aspect of food choice is not dietary protein or carbohydrate content, per se, but the same aspect as controls brain serotonin levels, i.e. the ratio of one nutrient to the other. Initially, we allowed rats to choose between two diets differing in CHO% and, simultaneously, in protein/CHO ratio. In the first two experiments, 12 possible diet pairs for diets containing 12, 32, 52, and 72% CHO were offered to rats, with CHO as either dextrin or sucrose and with diets either isoprotein/isofat (Experiment 1) or isoprotein/isocalorie (Experiment 2). In Experiment 3, 6 CHO diets ranging from 5-55% CHO (3.6-0.30 protein/CHO ratio) were paired with an isocaloric, isoprotein 65% CHO (0.28 ratio) diet. In Experiment 4, 6 CHO diets ranging from 5-65% CHO (3.6-0.28 ratio) were paired with an isocaloric, nonisoprotein 84% CHO (0.07 ratio) diet. Total food eaten was measured and grams of protein and carbohydrate eaten were calculated every 24 h.

The results of these experiments suggest that (1) caloric intake is regulated in deference to nutrient composition, at least with diets containing sufficient protein; (2) sucrose-fed rats consume more calories than dextrin-fed rats, emphasizing the need to consider the quality and not just quantity of the diet(s); (3) rats choose their foods so as to obtain a protein/CHO ratio ranging from 0.20-0.43, even when the possible range of ratios available to them is as wide as 0.07-3.6.

Pharmacological facilitators of serotoninergic mechanisms (fluoxetine, fenfluramine) have been shown to spare protein intake while decreasing caloric intake and, specifically, the self-selection for carbohydrate (Wurtman and Wurtman, Life Sci., 1979, 24, 895). The present study suggests that rats choose among available diets to obtain net protein/CHO ratios which will neither increase nor decrease brain serotonin.

264.2 INTESTINAL SATIETY IS ELICITED BY L- BUT NOT D-PHENYLALANINE. M.F. Lew*, J. Gibbs and G.P. Smith. Dept. of Psychiatry, Cornell Univ. Med. Coll. and E.W. Bourne Behav. Res. Lab., The New York Hospital, White Plains, NY 10605.

When rats provided with chronic gastric cannulas sham feed a liquid food following an overnight food deprivation, satiety is absent (Young et al, 1974). If a small amount of liquid food is placed directly into the small intestine of sham feeding rats, satiety reappears (Liebling et al, 1975). The mechanism(s) of this intestinal satiety are unknown, but we have postulated that endogenous peripheral circulating cholecystokinin (CCK) plays a role (Antin et al, 1978). Consistent with this hypothesis, gastric preloads of the L-isomer of phenylalanine (L-phe, a potent CCK releaser--Meyer & Grossman, 1972) reduce food intake in the intact rhesus monkey (Gibbs et al, 1976) and intact rat (Anika et al, 1977), while gastric preloads of the D-isomer (D-phe, a weak CCK releaser) do not reduce food intake in either species. To avoid the confound introduced by gastric preloads (activation of both gastric and intestinal satiety mechanisms), we tested the satiety potencies of L-phe and D-phe when they were delivered directly to the small intestine of sham feeding rats.

Five adult male Sprague Dawley rats were implanted with chronic stainless steel gastric cannulas and with chronic Silastic duodenal catheters, with tips 2.5 cm distal to the pylorus. After an overnight, 17-h deprivation of maintenance food (Purina pellets), gastric cannulas were opened and rats were offered a liquid food (50% BioServ) for sham feeding during a 1-h test period. Beginning 12 min after food presentation, a 3% solution of L-phe or D-phe, or of 0.15M NaCl as the vehicle control, was infused via duodenal catheters, at a rate of 1.2 ml-min^{-1} for 8 min. Sham feeding rates were recorded at 5 min intervals.

During the 40 min immediately following infusion on days when rats received intraduodenal 0.15M NaCl, they consumed a total of 70.6±6.2 ml (mean±SEm) at a relatively stable rate which ranged from 6.9±0.8 to 8.9±0.6 ml per 5-min interval. On days when rats received L-phe, they consumed 18.6±7.2 ml (difference $p < 0.005$ vs NaCl) at rates ranging from 0.6±0.6 ml to 3.8±1.5 ml. The latency to the onset of L-phe action was rapid: significant inhibition appeared before L-phe infusion ended. On days when rats received D-Phe, they consumed 58.2±12.3 ml (difference not significant vs NaCl) at rates ranging from 5.6±1.5 to 7.4±0.8 ml.

The results show that an intestinal load of L-phe produces a rapid and potent suppression of sham feeding, while an equivalent load of D-phe has no significant effect. We interpret these findings as additional evidence supporting a potent role for endogenous CCK in mediating intestinal satiety.

Support: RSDA #MH70874 and Hirschl Career Scientist Award (JG); RSA #MH00149 and #MH15455 (GPS).

264.3 THE EFFECTS OF INTRAVENTRICULAR GLYCEROL INFUSION ON FOOD AND WATER INTAKE AND BODY WEIGHT IN YOUNG FEMALE RATS.
J. K. Nishita*, E. H. Ellinwood, Jr., W. J. K. Rockwell* (SPON: H. K. H. Brodie). Dept. of Psychiatry, Duke University Medical Center, Durham, North Carolina 27710.

Glycerol has been proposed as a humoral signal to the brain that relates the state of adipose tissue mass and its utilization. A variety of peripheral and central techniques have shown that glycerol can suppress food intake and reduce body weight in adult rats. Furthermore, these studies have shown that short-term administration of glycerol (7 days) produce transient effects on food intake and body weight; returning to normal levels within 3-4 days (Grinker et al., 1980). However, long-term glycerol treatment (13 days) may produce a permanent reduction in body weight but not in the rate of weight gain (Wirtshafter & Davis, 1977).

This study measured the effects of continuous short- and long-term intraventricular infusion of glycerol on body weight and 24 hour food and water intake in young female rats (37 days). Rats were implanted with 25 ga midline cannula aimed at the third ventricle. Following recovery and baseline measurements of body weight and ingestion, rats were implanted subcutaneously with Alzet osmotic pumps containing either 0.15 M glycerol at an osmolar concentration of 300 milliosmoles/l, or 0.15 M saline. Short-term infusions were made through a 32 ga cannula at a rate of 1.0 ul/hr for 7 days and long-term infusions were at a rate of 0.5 ul/hr for 14 days. Body weight and food and water intake were recorded daily during the infusion and for 10 days following pump removal. All rats then received 7 days of glycerol injections (40 mg/kg) followed by 7 days of no injection.

Glycerol produced reductions in body weight and food intake during both short- and long-term continuous intraventricular infusions. Both short- and long-term treated rats also showed a reduction in the rate of weight gain during infusion. Peripheral glycerol injections produced a more pronounced effect in glycerol-infused rats then in controls. These results suggest that intraventricular glycerol infusion in young maturing female rats may produce long-term effects in terms of sensitivity to glycerol.

Grinker et al., Brain Res. Bull., 5, Suppl. 4, 29-35, 1980.
Wirtshafter & Davis, Science, 198, 1271-1274, 1977.

264.4 Chronic Meal-Contingent Infusion of Insulin into Free-Feeding Rats Can Produce Hyperphagia and Accelerate Growth Rate.
D.B. West, K. Parmely, D.J. Braget and S.C. Woods. Department of Psychology, University of Washington, Seattle, WA 98195.

Bilateral lesions of the ventromedial hypothalamus (VMH) in rats leads to hyperphagia, hyperinsulinemia and obesity. VMH lesions also result in an exaggerated secretion of insulin during a meal. Pancreatic denervation by either vagotomy or transplantation of a pancreas into rats with chemically destroyed islets has been reported to block both this heightened insulin secretion and the obesity produced by destruction of the VMH. This suggests that neurally-mediated hypersecretion of insulin during feeding may contribute to the development of obesity in VMH-lesioned rats.

In order to test this hypothesis, adult, female Sprague-Dawley rats (n=5) were implanted with intraperitoneal catheters and habituated to computer-monitored enclosures which recorded food intake by a pellet detecting eatometer. Initially, at the beginning of each spontaneous meal, 0.27 ml of physiological saline was infused over a period of 150 seconds into 3 of the rats. The other 2 rats were given an equivilent number of random infusions by yoking their infusions to rats receiving meal-contingent drug administration. Saline infusion did not affect growth rate or food intake in either of the groups. When 27 mU of purified porcine insulin (INS, Nordisk) was infused at the start of each spontaneous meal over a 4 day period, food intake and rate of growth of both groups remained unaffected. With meal-contingent infusion of 135 mU of INS for 6 days, however, total daily food intake increased by an average of 26.9% (± 2.1) compared with baseline intake, and growth rate increased from 0.3 (±0.6) to 1.2 (± 0.6) g/day. This increase of food intake with INS infusion was due to larger meals (1.8 ± 0.1 vs 1.3 ± 0.1 g/meal) and not due to a change in feeding frequency (13.4 ± 1.6 vs 13.6 ± 1.3 meals/day) Rats receiving random infusions also increased their total daily food intake by 31.6% (± 0.7) and increased their growth rate from 0.3 (± 0.3) to 2.4 (±1.1) g/day. This increased food intake was due to more meals (14 3 ± 0.1 vs 12.2 ± 0.3 meals/day) and not due to larger meals (1.5 ± 0.4 vs 1.5 ± 0.3 g/meal).

These data support the notion that chronic enhancement of insulin secretion during a meal can lead to hyperphagia and obesity. The rather large dose of insulin infused in this study may have produced plasma values beyond the normal range for VMH rats. The observation that rats receiving random infusions of INS also increased food intake, but did this by increasing frequency of feeding, suggests that the hyperphagia of both groups may be due to insulin-induced hypoglycemia.

264.5 SYMPATHETIC BLOCKADE ALTERS MEAL PATTERN.
L.A. Campfield* and F.J. Smith*. (SPON: R.C. ROGERS). Depts. of Physiology and Engineering Sciences, Northwestern University, Chicago, IL 60611 & Evanston, IL 60201 and College de France, Paris.

Differential insulin secretory responses following dark and light phase meals suggest that prandial insulin response, and its autonomic modulation, may be an important variable in the dual periodicity of feeding. The role of sympathetic control of insulin secretion in the neuroendocrine mechanisms underlying feeding periodicity was examined using phentolamine, an alpha adrenergic blocking agent. Female Wistar rats were implanted with chronic femoral cannulas and were maintained on a 12 hr light/dark cycle with free access to powdered food and water. Food intake was continuously recorded by monitoring food cup weight. Following complete recovery, two studies were performed. In the behavioral studies, each rat received infusions of either isotonic saline or phentolamine (0.5 mg) at a rate of 3 µl/min during the last seven hours of the light phase on alternate days. Sixteen saline and eleven phentolamine trials were performed in five rats. Meals were defined using a 40 min criterion. Following phentolamine infusion, the size of the first dark phase meal was increased by 76±25% compared to saline infusion. The onset ratio of the second meal was also significantly increased (70±28%) following phentolamine. However, other parameters of the dark and light phase meal patterns as well as total food intake and body weight were not altered. Increased meal size and onset ratio observed early in the dark phase following phentolamine were typical of those observed at the end of the dark phase in saline treated rats. In the metabolic studies, identical infusion protocols were used except food was removed 2 hr prior to infusions. Three blood samples were collected from lightly heparinized, conscious rats prior to the beginning and the end of the saline or phentolamine infusion and 2 hr post infusion. Plasma glucose concentrations were similar after both infusions. However, plasma insulin concentration significantly increased (2.1±.6 vs 0.6±.1 ng/ml) during the phentolamine infusion returning to levels observed in saline treated rats within 2 hr post infusion. These data demonstrate that phentolamine infusion during the last portion of the light phase resulted in: 1) elevated plasma insulin concentrations; 2) increased size of the first dark phase meal; and 3) increased onset ratio of the second dark phase meal. These results suggest that elevation of insulin concentration during the last portion of the light phase may induce a shift in subsequent dark phase meal pattern. Therefore, the sympathetic nervous system, through its inhibitory action on insulin secretion, has the potential to modulate feeding periodicity.

264.6 EFFECTS OF CHOLECYSTOKININ ON RUNWAY PERFORMANCE.
J. E. Cox*, R. J. Toney*, and D. J. Wiebe* (SPON: C. Oyster). Dept. Psychology, University of Alabama in Birmingham, Birmingham, AL 35294.

Injections of cholecystokinin (CCK) reduce food intake. A prevalent interpretation is that injected CCK mimics a satiety-producing action of endogenous CCK, but many uncertainties remain as to the motivational sequelae of the injection. We investigated effects of CCK on appetitive motivation by examining runway performance for food reward after CCK injections.

Male, Sprague-Dawley rats (approximately 350 g) were trained to traverse a 4 ft runway for food reward after 20 hr food deprivation. Reinforcement was 0.2 ml of 10% sucrose. Two sequences of injection of CCK-octapeptide (Kinevac, Squibb) were then administered. In each case i.p. drug injections alternated on consecutive days with equivolemic doses of normal saline in a randomized cross-over design. Injections were given 10 min prior to the test trial on that day. In the first sequence, 6 rats received 5 doses of CCK-octapeptide. Mean (±SE) running speeds (ft/s) were:

	0.5	1.0	2.0	4.0	8.0 µg/kg
Saline	1.82±.19	1.43±.18	1.60±.20	1.73±.10	1.70±.09
CCK	1.90±.07	1.40±.20	1.40±.18	1.37±.14	1.45±.06

Analysis of variance (ANOVA) revealed no significant effects of Drug, Dose, or the interaction of Drug x Dose. Thus, CCK did not reliably alter running speed under these conditions.

In the second series of injections, 4 rats used above were given 2.0 and 8.0 µg/kg CCK alternating on consecutive days with saline injections. For these trials rats were allowed 30 min access to 10% sucrose once they reached the goal box. Percentage suppression of intake (compared to saline trials) after 2 and 8 ug/kg CCK was 29.0±5.7% and 70.0±6.7%, respectively ($p < .05$ for both doses compared to saline). Corresponding decreases in running speed were -10.0±13.7% and 30.8±9.0%. Thus, running speed was not at all reduced by 2.0 µg/kg CCK and decreased by almost one-third at 8.0 µg/kg. The latter effect was marginally significant ($p < .10$).

These results show that CCK-octapeptide is effective in significantly suppressing food intake at doses at which no slowing of runway performance is seen (2 µg/kg). Since running speed varies as a function of hunger and incentive value of the reinforcement (Bolles, 1975), CCK can apparently reduce intake in the absence of changes in these appetitive factors. This outcome is consistent with the proposal that motivational effects of CCK operate later in the appetitive sequence, perhaps interacting with food intake to enhance signals of satiety. However, because CCK may reduce running speed at higher doses (8 µg/kg), further decreases in feeding seen with increasing doses may be a function of additional factors.

264.7 CCK-8 DECREASES BODY WEIGHT IN ZUCKER RATS. R.G. Campbell* and G.P. Smith. Dept. of Psychiatry, Cornell Univ. Med. Coll. and E.W. Bourne Behav. Res. Lab., New York Hospital, White Plains, NY 10605

Cholecystokinin (CCK) has been shown to decrease meal size in animals and humans. CCK has not, however, been shown to produce body weight loss. We report here the results of chronic treatment with CCK on body weight in the genetically obese Zucker rat.

In the first experiment 10 genetically obese female Zucker rats were adapted to a 12 h reverse light/dark cycle with the dark phase beginning at 0700 h. The rats were restricted to three 0.5 h meals per day (0930-1000, 1230-1300 and 1530-1600) of Purina powder on Monday through Friday with tap water available at all times. On Friday at 1630, rats were given free access to twice the weight of powder they ate during the three Friday meals.

Following a five week diet restriction phase, rats were randomly assigned to control and experimental groups. The experimental group received intraperitoneal injections of CCK-8 (6 mcg-kg^{-1}) dissolved in 0.15M NaCl while the control group received intraperitoneal injections of 0.15M NaCl. Both groups received their injections prior to each meal for a three week period. Food intake(g) was measured for each meal and body weight(g) was measured Monday through Friday at 0900. A replication of the first experiment was done with eleven obese male Zucker rats.

CCK increased body weight loss and decreased food intake in both experiments without producing signs of toxicity.

Treatment	Body Weight[a]	Loss of Weight: Actual(g)	Loss of Weight: %Initial	Food Intake[b](g)
Experiment 1				
CCK (n=5)	422.6 ± 11.3	58.2±4.1**	13.9±1.2*	69.0±4.4*
Saline (n=5)	417.8 ± 31.7	33.6±5.7	8.6±1.9	107.4±11.7
Experiment 2				
CCK (n=6)	527.0 ± 19.3	77.2±6.6***	14.6±1.0***	70.5±5.0***
Saline (n=5)	529.6 ± 14.9	25.6±5.3	4.9±1.0	131.3±7.5

*p < .05, **p <.01, ***p <.001

[a]mean body weight prior to treatment
[b]mean food intake for Tue, Wed, and Thurs meals during three week treatment period.

We conclude that chronic CCK treatment in conjunction with a restricted diet enhances body weight loss in genetically obese Zucker rats. The increased loss of body weight appears to be due to the decreased food intake.

These results support the therapeutic potential of CCK for the treatment of obesity.

This research was supported by NIMH grants MH00149 and MH15455.

264.8 VAGAL AFFERENT AXONS MEDIATE THE SATIETY EFFECT OF CCK-8. G.P. Smith, C. Jerome* and R. Norgren (SPON: C. Pfaffmann). Cornell University Medical College, New York, NY 10021 and Penn State Univ. Col. of Medicine, Hershey, PA 17033

Bilateral abdominal vagotomy abolished or markedly reduced the satiety effect of peripherally administered CCK-8, but unilateral abdominal vagotomy did not. Since pharmacological "efferent vagotomy" by atropine methylnitrate did not block the satiety effect of CCK-8, we suggested that loss of vagal afferent axons was the critical lesion (Smith et al, 1981). To test this hypothesis, we unilaterally cut the afferent or efferent vagal rootlets intradurally as they approached the medulla. In a second operation, a unilateral abdominal vagotomy was performed that cut the abdominal vagal afferent and efferent fibers that project to and from the side of the medulla contralateral to the intradural section. The combination of cut afferent rootlets and unilateral abdominal vagotomy produced bilateral afferent abdominal vagotomy, but only unilateral efferent abdominal vagotomy. The combination of cut efferent rootlets and unilateral abdominal vagotomy produced bilateral efferent vagotomy, but only unilateral afferent vagotomy.

All rats were tested after 17 h food deprivation. CCK-8 (6 mcg-kg^{-1}, ip) or saline was administered just before rats were given a sweet milk diet and 30 min intakes were measured. After the food intake tests were completed, the afferent or efferent rootlet lesion was confirmed by the complete or almost complete loss of anterograde or retrograde HRP labeling in the medulla after application of HRP to the cervical vagus on the side of the rootlet lesion. The unilateral abdominal vagal lesion was verified anatomically under microscopic control. Six of 6 rats with verified bilateral afferent abdominal vagotomy failed to inhibit food intake after CCK-8, but 2 of 2 rats with verified bilateral efferent abdominal vagotomy and 1 surgical control showed the usual inhibition of food intake after CCK-8. These results confirm the hypothesis that vagal afferent axons mediate the satiety effect of peripherally administered CCK-8.

Support by grants MH15455, MH00149, and NS10150.

264.9 HEPATIC VAGOTOMY BLOCKS THE INHIBITORY EFFECT OF GLUCAGON, BUT NOT OF EPINEPHRINE ON FEEDING. N. Geary, M. Licht*, and L. Mac Isaac*. Department of Psychology, Columbia University, New York, NY 10027.

Peripheral administration of pancreatic glucagon or of epinephrine under appropriate conditions can inhibit feeding in rats. To investigate the involvement of the hepatic branch of the vagus nerve in these effects, we compared the potencies of glucagon and epinephrine to inhibit feeding in rats with selective vagotomis of only the hepatic branch of the abdominal vagus and in sham operated rats. Rats were maintained on tap water and ground Purina rodent chow under a reversed 12:12 LD cycle (lights off: 1200). On test days, rats were food deprived at 0900. At 1100 glucagon (100 mcg/kg), epinephrine (200 mcg/kg), or the vehicle solution alone (1 ml/kg 3% DMSO, 0.9% NaCl) was intraperitoneally injected and fresh food was presented. The rats were observed until they stopped feeding and retired to the rear of the cage to groom and rest. Meal size was then measured by weighing the food cups. Glucagon and epinephrine reduced meal size by comparable amounts in sham operated rats (median reduction as percent of control; epinephrine, 27%; glucagon, 20%; n=7). In hepatic vagotomized rats, however, the hormones had different effects. Hepatic vagotomy completely blocked glucagon's potency to inhibit feeding, but did not significantly change epinephrine's potency to inhibit feeding (median meal size reductions: epinephrine, 22%; glucagon, 5%; n=8). These data demonstrate that under conditions in which the hepatic vagus is necessary for pancreatic glucagon's satiety effect, it is not required for the anorexic effect of epinephrine. This does not support Russek and Racotta's (Front. Hormone Res. 6: 120-137, 1980) hypothesis that the effects of these two hormones on feeding are mediated by a common effect on hepatic metabolism

264.10 GLUCAGON'S SATIETY EFFECT IS ABOLISHED BY INTRAHEPATIC INJECTION OF ALLOXAN. B.Weick, S.Stone* and S.Ritter. College of Veterinary Medicine, Washington State University, Pullman, WA 99164-6520.

Work from several laboratories indicates that glucoreceptors capable of inhibiting food intake may reside in the liver. Furthermore, glucagon, a proposed satiety hormone, may exert its satiety effect by an action on the liver since hepatic vagotomy abolishes glucagon's satiety effect. Although the mechanism of glucagon's satiety effect is not known, it has been hypothesized that elevation of hepatic glucose subsequent to the release of glucagon during a meal might aid in the termination of feeding by activating hepatic glucoreceptors. We speculated that if glucoreceptors controlling feeding do in fact exist in the liver, they might be vulnerable to the toxic effects of alloxan, a substance which is toxic to some glucose sensitive cells in pancreas and brain. To test this idea, we injected a subdiabetogenic dose of alloxan (65 mg/kg) or vehicle solution (acidified saline, pH 3.0) directly into the hepatic portal system of rats in an attempt to damage the postulated hepatic glucoreceptors. The animals were then trained to drink a palatable liquid food (vanilla Instant Breakfast) and glucagon suppression of feeding was measured in a series of 30-min tests. On separate test days glucagon (400 or 800 ug/kg,i.p.) or vehicle injections were made 8 min after or 0, 4, 8, or 12 min prior to presentation of liquid food. Glucagon (800 ug/kg) significantly reduced food intake in controls when injected 0, 4 or 8 min prior to the test, but failed to suppress feeding in alloxan-treated rats. Under the most effective conditions glucagon suppressed intake in controls to 63.3 ± 19.3% of their baseline intake ($p < .01$), whereas alloxan-treated rats consumed 133.6 ± 19.3% of their baseline intake. Elevation of blood glucose during the 3 hr following glucagon was similar in both groups. In addition, cholecystokinin-induced satiety was not attenuated in alloxan-treated rats. The fact that alloxan abolished glucagon's satiety effect without impairing the ability of this hormone to elevate blood glucose suggests that hepatic-portal alloxan may damage a satiety mechanism which possibly operates by detection of glucose levels. This mechanism may involve hepatic vagal afferents, since hepatic vagotomy also abolishes glucagon satiety. If vagal afferents are damaged by hepatic-portal alloxan, the presumed damage must be limited to the hepatic innervation since cholecystokinin-induced satiety, which is mediated by the gastric vagus, was not impaired by this treatment. Finally, our alloxan treatment did not make animals diabetic or alter spontaneous weight gain. However, base-line intakes of the liquid food tended to be greater in alloxan-treated rats than in controls.

264.11 CCK-8 DECREASES SUCROSE INTAKE IN CHRONIC DECEREBRATE RATS. H.J. Grill, D. Ganster* and G.P. Smith (SPON: R. Murphy). Univ. of Pennsylvania, Philadelphia, PA 19104 and Cornell University Medical College, White Plains NY 10605.

Chronic decerebrate rats respond to the satiating effects of a gastric preload of nutrient by decreasing the intake of sucrose delivered through an intraoral catheter (Grill and Norgren, 1978). Such preloads stimulate preabsorptive and postabsorptive satiety mechanisms. CCK-8 is released by nutrients in the small intestine, and has a satiety effect in numerous animals and humans (Smith et al, 1981). To determine if endogenous CCK-8 released by a gastric preload could be one of the mechanisms that satiates the chronic decerebrate rat, we investigated the effect of exogenously administered CCK-8 on sucrose intake by chronic decerebrate rats.

Seven chronic decerebrate rats were prepared and maintained as described by Grill and Norgren (1978). After habituation to the test situation and the intraoral delivery of distilled water, decerebrate rats were tested in the light phase after 24 h of food deprivation. They received an ip injection of CCK-8 (6.3 to 8.3 $mcg\text{-}kg^{-1}$) or an equal dose of desulfated CCK-8 (which has no satiety effect in normal rats) just prior to the beginning of intraoral delivery of 0.1M sucrose at a rate of 0.82 $ml\text{-}min^{-1}$. Intraoral sucrose infusion was stopped when rats rejected the fluid. CCK-8 significantly decreased sucrose intake in all 7 rats: intake ($\bar{X}\pm SE$) after CCK-8 = 3.0±1.26 ml, intake after desulfated CCK-8 = 12.1±2.48 ml, $p< .02$. To compare the inhibition of sucrose intake by CCK-8 in decerebrate rats with the effect of CCK-8 in neurologically normal rats, we tested 6 normal rats under identical conditions. CCK-8 decreased intraorally delivered 0.1M sucrose intake in 5 of 6 normal rats: intake after CCK-8 = 6.4±1.34 ml, intake after desulfated CCK-8 = 19.7±3.78 ml, $p < .02$. These results demonstrate that the decerebrate rat has sufficient neural mechanisms to mediate the satiating effect of CCK-8 on sucrose intake and that endogenous CCK-8 could mediate part of the satiating effect of a gastric preload in decerebrate rats.

Supported by NSF grant BNS8210734 and NIMH grants MH00149 and MH15455.

PERIPHERAL AUTONOMIC NERVOUS SYSTEM II

265.1 PATHOLOGICAL DISINHIBITION OF A SOMATOSENSORY-SYMPATHETIC REFLEX IN INDIVIDUALS WITH IDIOPATHIC HYPERIDROSIS, P. Marchettini,* E. Torebjörk,* W. Culp and J. Ochoa. Dartmouth Medical School, Hanover, N.H. and University of Wisconsin, Madison, WI, U.S.A.

The syndrome idiopathic hyperidrosis consists of excessive, often unmotivated and embarrassing sweating of palms, soles and axillae. Individuals of all ages and races may be affected. The cause of the disorder remains unknown; it is accepted that the condition is not a manifestation of neurosis. Among the many therapies attempted, the most effective is sympathectomy.

Below spinal cord lesions in man, there remains spinal reflex sudomotor activity in response to intense cutaneous thermal stimulation. In addition, under similar circumstances, reflex sweating is observed with bladder or rectal distention or following tactile or irritative cutaneous stimulation. These observations suggest the existence in man of dormant spinal sudomotor reflex arcs with mechanoreceptor rather than thermoreceptor afferents.

Taking advantage of the microneurographic technique (see Vallbo, et al,[1]), and of pulse plethysmography, we have studied pathophysiological phenomena in 3 otherwise healthy individuals exhibiting idiopathic hyperidrosis. By recording directly the neural autonomic outflow with intraneural microelectrodes in skin and muscle nerve fascicles, while monitoring equivalents of skin vasoconstriction and observing gross palmar sweating, we have made observations which reveal that these subjects have a) an abnormal increase in postganglionic sympathetic efferent activity under basal conditions, and b) an abnormal sweat reflex drive from skin afferents. The available evidence indicates that the abnormal circuitry impinges on sympathetic sudomotor outflow to skin, whereas vasomotor outflow to skin and muscle are unaffected.

Since autonomic subsystems have independent pathways, selective dysfunction of the sudomotor system in idiopathic hyperidrosis is readily explained. Analogous dysautonomia selective to other subsystems, perhaps Raynaud's syndrome from selective skin vasomotor dysfunction, should be demonstrable with the techniques applied in the present study.

A specific disorder of inhibitory interneurons is postulated for idiopathic hyperidrosis.

References

1: Vallbo et al., (1979) Physiological Reviews, 59, 919-957.

265.2 SYNAPTIC TRANSMISSION IN THE CHRONICALLY DECENTRALIZED MIDDLE CERVICAL AND STELLATE GANGLIA OF THE DOG. J.A. Armour, Dept. of Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada.

Afferent stimulation of one canine thoracic cardiopulmonary nerve generates compound action potentials (CAPs) in the efferent axons of other, ipsilateral cardiopulmonary nerves. This can occur before and after acute decentralization of the middle cervice (MCG) and stellate (SG) ganglia (Armour, 1976). These CAPs can be modified by a number of pharmacological agents (Armour, 1983). It was proposed that thoracic autonomic reflexes function via synaptic mechanisms located primarily in the MCG. It is not known if such reflex arcs are dependent upon two or more neurons which are located in the thoracic autonomic nervous system. Can some of these reflex mechanisms occur via neurons which are only in thoracic autonomic ganglia? A left thoracotomy was performed in twelve dogs following anesthesia (ketamine and pentobarbital) and the initiation of artificial respiration with a positive pressure device. The thoracic neural elements were identified. Connections between the left stellate ganglion and the central nervous system were cut. The upper thoracic vagosympathetic complex was cut as well. Thus, the SG and MCG were decentralized. The chest was closed. Fourteen days later the animals were anesthetised (ketamine and ∝chloralose) and the left thoracic autonomic nerves and ganglia exposed. The left cranial medial, intermediate medial, caudal medial and ventral lateral cardiopulmonary nerves were desheathed under oil and laid over bipolar stimulating and recording electrodes. Stimulation (0.5 Hz, 2-4 msec, 4-8 V) of one nerve generated CAPs in another ipsilateral nerve. In some experiments CAPs were modified by alteration of stimulation frequency or by the pharmacological agents hexamethonium, atropine, phentolamine and propranalol. In all experiments, CAPs were altered by local injections of chymotrypsin or manganese into the MCG (Armour, 1983). Injections of similar volumes of normal saline into the same regions had little, if any, effect on the CAPs. It is concluded that synaptic activity in thoracic autonomic ganglia generated by afferent nerve stimulation persists in chronically decentralized thoracic autonomic ganglia and that such activity can be modified by altered stimulation frequency and by pharmacological agents. It is proposed that afferent and efferent neurons exist in the thoracic autonomic nervous system which can function independently from the central nervous system.

(Supported by grants from the MRC and N.S. Heart Foundation).

265.3 PRE- AND POSTGANGLIONIC SYMPATHETIC PATHWAYS TO MESENTERIC ARTERIES IN THE GUINEA PIG. D. L. Kreulen, Dept. of Pharmacology, University of Arizona, College of Medicine, Tucson, AZ 85724.

The postganglionic mesenteric nerves emanating from the inferior mesenteric ganglion (IMG) of the guinea pig contain both pre- and postsynaptic nerve fibers. The purpose of these studies was to determine the contribution of the various ganglionic pathways to the neural outflow to mesenteric arteries. In vitro preparations consisting of the IMG with attached preganglionic [lumbar splanchnic (LSN)], postganglionic or mesenteric [lumbar colonic (LCN) and hypogastric (HGN)] and intermesenteric (IMN) nerves and mesenteric blood vessels (inferior mesenteric artery) were dissected from male guinea pigs and pinned to the floor of a recording chamber (Krebs, 37°C). Intracellular responses to stimulation of para-arterial nerves were recorded in smooth muscle cells of the inferior mesenteric artery and its secondary and tertiary branches. The recording sites were 10-15 mm away from the stimulating sites. Membrane potentials ranged from -70 to -85 mV. In response to single nerve shocks excitatory junction potentials (EJPs) were recorded in arterial smooth muscle cells; their amplitude ranged from 1-11 mV. All EJPs were blocked by tetrodotoxin (10^{-6}M) but not by phentolamine (10^{-6}M). For each arterial cell, the averaged EJP amplitudes (20-50 responses) were compared for each of the four nerve fibers. All cells studied gave responses to LCN (postganglionic) and LSN (preganglionic) stimulation, 40% to IMN stimulation and 22% to HGN stimulation. EJPs in response to LCN stimulation were always of the greatest amplitude. The amplitude of EJPs in response to LSN stimulation were 36% (± S.E.3%, n=15) of the EJPs in response to LCN, to IMN stimulation they were 38% (± S.E.12%, n=4) and to HGN stimulation they were 12% (n=2). IMN stimulation usually gave two EJPs of different latency in response to each stimulus. Repetitive nerve stimulation (2 Hz) resulted in an increase of EJP amplitude and a slow depolarization of the membrane potential. This facilitation was greater for stimulation of LSN than for LCN, indicating the additional facilitation at ganglionic synapses. Phentolamine (10^{-6}M) depressed the slow depolarization but increased the amount of facilitation. These studies demonstrate the participation of several ganglionic pathways in the neural control of mesenteric blood vessels. They also show that the IMG does not relay all presynaptic impulses to postganglionic fibers innervating the blood vessels. The activation of outflow to the vessels by HGN and IMN stimulation suggests a reflex pathway through the ganglia from the regions supplied by these nerves. *Support HL 27781.*

265.4 NEURONS WITH HIGH-AFFINITY GABA-UPTAKE SITES IN THE MYENTERIC PLEXUS OF THE GUINEA-PIG, RAT AND CHICKEN Saffrey, M.J.*, Marcus, N.*, Jessen, K.R.* & Burnstock, G.* (SPON: Anna B. Drakontides, Ph.D.)
Department of Anatomy & Embryology and Centre for Neuroscience, University College London, Gower Street, LONDON WC1E 6BT.

Autoradiographic techniques were first used to localize putative GABAergic (gamma-aminobutyric acid) neurons in the myenteric plexus of the guinea-pig caecum by Jessen et al. (Nature, 281:71, 1979), and subsequently in that of the ileum, by Krantis & Kerr (Neurosci. Lett., 23: 263, 1981). In order to determine whether such neurons are found throughout the enteric nervous system, we have examined the myenteric plexuses of the guinea-pig proximal and distal colon, the rat proximal colon and the chicken gizzard for the presence of neurons which take up ^{3}H-GABA.

Plexuses were isolated from the gut wall after enzyme treatment, and then incubated in 1.4×10^{-8}M ^{3}H-GABA and 10^{-3}M ^{3}H-GABA and 10^{-3}M Beta-alanine either immediately, or after maintenance in tissue culture for 7-14 days. Control preparations were incubated in the presence of 2,4-DABA, (2,4-diaminobutyric acid) a specific neuronal GABA uptake inhibitor. After incubation for 20 minutes at room temperature, the preparations were fixed, dried and processed for autoradiography.

Scattered neurons selectively labelled with ^{3}H-GABA were found in the myenteric plexuses from all the areas examined; control preparations contained no labelled neurons. these results show that neurons with high-affinity GABA uptake sites are widespread in the enteric nervous system. Recent biochemical and pharmacological evidence for GABA as an enteric neurotransmitter is discussed.

265.5 THE EFFECTS OF HYPOXIA-INDUCED STRESS ON CATECHOLAMINE CONTENT AND SYNTHESIS IN THE RAT SUPERIOR CERVICAL GANGLION. J.J. Brokaw and J.T. Hansen. Department of Anatomy, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284.

In addition to norepinephrine (NE)-containing principal neurons, the rat superior cervical ganglion (SCG) also contains numerous small intensely fluorescent (SIF) cells which store predominantly dopamine (DA). These paraneurons are thought to act as interneurons and/or in an endocrine capacity to modify ganglionic transmission. Hypoxia is reported to stimulate activity in other catecholamine (CA)-containing tissues such as the adrenal medulla, the carotid body and abdominal paraganglia. We wished to examine the effects of hypoxia-induced stress on CA content, turnover, and in vivo tyrosine hydroxylase activity in the rat SCG using high performance liquid chromatography with electrochemical detection. The steady-state SCG concentrations of NE and DA were 174.2 ± 4.7 and 17.8 ± 0.7 pmol/ganglion, respectively. Animals were rendered severely hypoxic by placing them in an enclosed chamber flushed with 5% O_2 - 95% N_2. Hypoxic exposure for 30 min resulted in a 128% increase in DA over control levels ($p < 0.01$). No change was seen in NE content. Turnover was determined by administering a selective inhibitor of tyrosine hydroxylase (α-methyl-p-tyrosine, 250 mg/kg, ip) and measuring the time-dependent decline in CA levels. The half-lives of SCG NE and DA were 1.6 hrs and <0.5 hrs, respectively. The turnover of NE was not altered by hypoxia, but DA turnover was too rapid to assess any changes by this method. Therefore, accumulation of DOPA following the blockade of L-aromatic amino acid decarboxylase by NSD-1015 (100 mg/kg, ip) was used as an in vivo index of tyrosine hydroxylase activity, another measure of synthetic capacity. DOPA levels in SCG from rats exposed to 30 min of hypoxia were increased 65% above air-breathing control levels ($p < 0.05$). Though the exact location of this increased activity cannot be ascertained, the hypoxia-induced increase in DA content, coupled with the inability of hypoxia to alter NE content or turnover, suggests that hypoxia may activate the relatively small population of SIF cells. (Supported by USPHS grants HL-25508 and KO4 HL-00680 to J.T.H.)

265.6 MUSCARINE INCREASES TYROSINE HYDROXYLASE ACTIVITY AND PHOSPHOLIPID METABOLISM IN THE SUPERIOR CERVICAL GANGLION OF THE RAT. J. Horwitz*, S. Tsymbalov*, and R.L. Perlman* (Spon: J. Townsel).
Department of Physiology and Biophysics, University of Illinois College of Medicine, Chicago, IL 60680

Muscarinic agonists increase DOPA accumulation and produce a stable activation of tyrosine hydroxylase (TH) in the superior cervical ganglion of the rat. These agents also increase phospholipid turnover in this tissue. Muscarine (100 μM) typically causes a 2 to 3-fold increase in DOPA synthesis and a 7 to 15-fold increase in the incorporation of $^{32}P_i$ into phosphatidylinositol (PI) in the ganglion. We have studied the relationship between muscarine-stimulated phospholipid turnover and the muscarine-induced activation of TH. Both of these actions are apparent within two min of incubation with muscarine, and both are independent of extracellular Ca^{2+}. Although muscarine causes a much larger increase in phospholipid labelling than in DOPA synthesis, the two processes show a similar dependence on muscarine concentration; muscarine produces half-maximal increases in both processes at a concentration between 1 and 10 μM. Muscarine is more potent and more effective than bethanechol both for the stimulation of DOPA synthesis and for the stimulation of phospholipid metabolism. Thus, the effects of muscarinic agonists on phospholipid turnover and on DOPA synthesis appear to be mediated through a single class of muscarinic receptors.

When ganglia are preincubated with [^{3}H]inositol to label inositol-containing phospholipids and then incubated in the presence of Li^+ (an inhibitor of inositol 1-phosphatase), muscarine increases the accumulation of inositol 1-phosphate in the ganglia. The primary effect of muscarine on phospholipid metabolism may be to increase the breakdown of inositol-containing phospholipids. Several lines of evidence support the idea that there may be a causal relationship between the muscarinic stimulation of phospholipid metabolism and the muscarine-induced activation of TH. Li^+ inhibits the muscarine-induced increase in DOPA accumulation. Other agents that promote phospholipid hydrolysis, including phospholipase C and deoxycholate, also increase DOPA synthesis in the ganglion [control, 121±11 pmol/ganglion/hour; phospholipase C (B. cereus, 7.5 units/ml), 749±130; deoxycholate (1 mM), 359±21]. Phospholipase C, like muscarine, causes a stable activation of TH in the ganglion. Changes in phospholipid metabolism may mediate the activation of TH by muscarinic agonists.

Supported in part by research grant HL 29025 from the National Institutes of Health. J.H. is the recipient of NRSA HL06701 from the National Institutes of Health.

265.7 PROTEIN PHOSPHORYLATION IN THE SUPERIOR CERVICAL GANGLION. Anne L. Cahill* and Robert L. Perlman* (SPON: R. Greenberg). Dept. of Physiology and Biophysics, University of Illinois College of Medicine, Chicago, IL 60680.

Cholinergic agonists, cyclic AMP analogues, and veratridine have all been shown to increase dopa synthesis in the superior cervical ganglion (SCG) of the rat. These agents all appear to produce a stable activation of tyrosine hydroxylase (TH) in the ganglion (Horwitz and Perlman, unpublished observations). We have investigated the role of protein phosphorylation in mediating the actions of these agents in the SCG. Intact ganglia were incubated in vitro in media containing $^{32}P_i$. Following this incubation, ganglionic proteins were separated by two-dimensional electrophoresis; phosphoproteins were identified by radioautography of the dried gels. Thirty-two phosphorylated polypeptides, ranging in apparent molecular weight (M_r) from 18,000 to 135,000 and in isoelectric point (pI) from 4.5 to 7.0, were consistently seen. Two of these phosphorylated polypeptides were identified as TH on the basis of their electrophoretic identity with immunoprecipitated TH. These two TH polypeptides migrated as a closely spaced doublet with M_r of about 60,000 and pI of approximately 6.1. Incubation of the ganglia with 50 μM veratridine, 100 μM dimethylphenylpiperazinium (DMPP), 150 μM muscarine, or 1 mM 8-bromo cyclic AMP increased the incorporation of $^{32}P_i$ into the two TH polypeptides. Veratridine, DMPP and muscarine also increased the incorporation of $^{32}P_i$ into six other polypeptides. The M_r (pI in parentheses) of these other phosphorylated polypeptides were 67,000 (5.0), 59,000 (5.7), 21,000 (5.6), 21,000 (5.8), 19,000 (5.0), and 19,000 (5.1). Veratridine caused the greatest increase in incorporation of $^{32}P_i$ into these polypeptides, followed by DMPP and then muscarine. 8-Bromo cyclic AMP also increased the incorporation of $^{32}P_i$ into the 59,000 (5.7) polypeptide, but not into the other peptides listed. Instead, 8-bromo cyclic AMP increased the incorporation of $^{32}P_i$ into 45,000 (6.4) and 43,000 (6.3) polypeptides. Thus, these various agents all increase protein phosphorylation in the SCG. 8-Bromo cyclic AMP presumably activates a cyclic AMP-dependent protein kinase in the ganglion; veratridine and cholinergic agonists may activate a cyclic AMP-independent protein kinase(s). TH can apparently be phosphorylated by both the cyclic AMP-dependent and the cyclic AMP-independent protein kinases. The stimulation of TH phosphorylation by cholinergic agonists, cyclic AMP analogues, and veratridine may underlie the effects of these agents on dopa synthesis in intact ganglia. (This work was supported by NIH Research Grant HL29025.)

265.8 HORMONAL REGULATION OF PEPTIDES IN PERIPHERAL SYMPATHETIC NEURONS. R.W.Hamill, L.C.Terry, L.A.Guernsey* and M.Zorza*. Monroe Community Hospital/Univ Rochester Med Ctr, Roch, N.Y. 14603 and V.A. Hospital/Univ Michigan, Ann Arbor, Michigan 48105

Previous investigations indicate that hormonal factors regulate the catecholaminergic neurotransmitter synthesizing enzyme tyrosine hydroxylase (T-OH) in peripheral sympathetic ganglia. One month after bilateral castration in male Sprague-Dawley rats T-OH activity declines 75% in the hypogastric ganglion (HG), a terminal noradrenergic ganglion which innervates androgen responsive pelvic sex organs. Recent studies indicate that neuropeptides are distributed throughout the neuraxis and may coexist with catecholamines in peripheral sympathetic ganglia. For instance, the undecapeptide substance P (SP) and the tetradecapeptide somatostatin (SRIF) are present in ganglia and may share regulatory influences with catecholamines. The present studies were designed to extend our earlier investigation and were based on the hypothesis that gonadal steroids may regulate peptidergic as well as catecholaminergic characteristics of peripheral ganglia.

Substance P-like immunoreactive (SPLI) and somatostatin-like immunoreactivity (SLI) were measured by radioimmunoassay in the hypogastric ganglia (HG) of male Sprague Dawley rats 2,4 and 10 weeks following castration. SPLI decreased significantly($p<.05$) by 2 weeks (28%) and remained so at 4 (22%) and 10 (21%) weeks. To substantiate the specificity of testosterone in these alterations, castrated and sham-operated animals were administered testosterone decanoate (20mg/kg) or vehicle. Testosterone restored HG SPLI to normal levels. In order to examine whether these effects of gonadal steroids were restricted to SP or whether other neuropeptides are influenced similarly, SLI was measured in the HG. There was no change in HG SLI whether examined 2 weeks (control 46.6±6.6 pg/gang; castrated 44.3±4.3 pg/gang) or 8 weeks (control 59.1±5.6 pg/gang; castrated 62.7±7.1 pg/gang) after castration. To define whether this regulatory influence of hormonal factors exist in other sympathetic ganglia, SPLI and SLI were examined in the pelvic ganglion another pelvic autonomic ganglion. Castration had no effect on either SPLI (control 411.2± pg/gang; castrated 414.8±36.4 pg/gang) or SLI (control 220.3±13.4 pg/gang; castrated 176.8±21.4 pg/gang) when examined 10 weeks after castration. These studies indicate that SP but not SRIF in specific peripheral sympathetic ganglia is under gonadal (androgenic) influences. Taken together with earlier studies, these observations suggest that catecholaminergic and specific peptidergic systems in sympathetic neurons are under hormonal control.

Supported by MCH/ U of R research fund, AM-28443 and VA Merit Review Grant.

265.9 ON THE SUBCELLULAR DISTRIBUTION OF TYROSINE HYDROXYLASE IN BOVINE ADRENAL MEDULLA AND THE LOCUS OF EFFECT OF ACH IN SITU. J.W. Haycock#, R.J. George and J.C. Waymire. Dept. Neurobiology & Anatomy, University of Texas Medical School, Houston, TX 77225.

Acetylcholine (ACh), the physiological agonist of chromaffin cells, induces catecholamine secretion and a compensatory acceleration of catecholamine biosynthesis from bovine adrenal medulla. One mechanism for this compensatory increase in synthesis is the phosphorylation and activation of tyrosine hydroxylase (TH)[1], the first step in catecholamine biosynthesis. We have previously shown that a calcium-dependent kinase activity in the soluble fraction of medullary chromaffin cells is capable of mimicking the effects of ACh in situ[2]; however, recently it has been reported that TH and kinases capable of phosphorylating TH are present in bovine adrenal medullary chromaffin granule membranes[3]. Thus, the present studies investigated the subcellular locus of ACh's effects upon the phosphorylation of TH in purified, cultured chromaffin cells from bovine adrenal medullae.

First, we investigated the subcellular distribution of TH activity in both adrenal medullae and chromaffin cells purified from medullae. As reported by several other authors, a large portion of the TH activity (40-50%) from adrenal medullae was found in particulate fractions. However, with several different sonication media, over 90% of the TH activity associated with isolated chromaffin cells was soluble.

In addition, after labeling the cells with $^{32}P_i$, over 90% of the ^{32}P-TH was found in the soluble fractions. Stimulation of the cells with ACh increased the phosphorylation of TH but, again, over 90% of the increase was associated with soluble TH.

In an attempt to optimize detection of TH phosphorylation, crude homogenates, supernatant and particulate fractions from the chromaffin cells were prepared and phoshorylated in vitro in the presence of [gamma-^{32}P]ATP. Well over 90% of the phosphorylation of TH (and the stimulation thereof by cAMP and calcium) observed in the crude homogenate was also observed in soluble fractions. On the other hand, phosphorylation of a band which comigrated with TH in SDS-PAGE from particulate fractions was detectable only with very long autoradiographic exposures.

These data suggest then that although TH (and possibly kinases) can associate with particulate fractions when isolated from crude bovine adrenal medullae, the physiological effects of ACh are predominantly associated with soluble pools of TH and its attendant kinases.

[(1) J. Biol. Chem. 257, 12641 (1982); (2) Soc. Neurosci. Abstr. 8, 817 (1982); (3) J. Neurochem. 40, 661 (1983); (#) Presently at Rockefeller University, New York, NY 10021.]

265.10 GLUCOCORTICOID RECEPTORS AND THE REGULATION OF PNMT ACTIVITY IN CULTURED ADRENAL MEDULLARY CELLS. K.L. Kelner and H.B. Pollard. Lab of Cell Biology and Genetics, NIADDK, NIH, Bethesda MD 20205.

The activity of the adrenal medullary enzyme, phenylethanolamine N-methyl transferase (PNMT), is dependent on glucocorticoids secreted from the adrenal cortex. After withdrawal of glucocorticoid in vivo and subsequent loss of PNMT activity, the enzyme is known to require extremely high concentrations of glucocorticoids for reappearance of the activity. We are able to maintain isolated adrenal medullary cells in culture and measure PNMT levels in aqueous extracts of these cells. We could, therefore, ask whether PNMT activity in vitro was dependent on glucocorticoids and if so, whether high concentrations were necessary for activation. Maintenance of medullary cells in DMEM supplemented with 5% fetal calf serum and periodic measurement of PNMT indicates that after the removal of the cells from the animal, the specific activity of PNMT declines. There is an initial rapid decline in activity followed by a slower phase of loss of activity which approaches zero after 20-25 days in culture. PNMT activity can be increased 25 - 75% by glucocorticoids for up to 21 days in culture. Extensive dose response curves show maximal induction of PNMT at 10 μM dexamethasone in the presence of 5% fetal calf serum. Under these conditions maximal hydrocortisone stimulation occurs at 100 μM. In a serum free defined medium, maximal induction occurs at much lower concentrations of dexamethasone (10 nM) and hydrocortisone (10 nM). This may be due to the difference in availability of free steroid in a serum free defined medium and a 5% serum medium which contains the serum glucocorticoid binding proteins. Thus, unlike in vivo, in an in vitro situation adrenal medullary cells are capable of responding to very low concentrations of steroids.

Measurements of the affinity of the cytoplasmic glucocorticoid receptor indicate that, as expected, it has an extremely high affinity for its ligands. The K_d is 1.3 nM which is similar to the concentration of glucocorticoid needed for half maximal stimulation of PNMT. Therefore, the isolated adrenal medullary cell lacks the mechanism by which the organized adrenal medullary tissue maintains its apparent low sensitivity to glucocorticoids.

265.11 REGULATION OF CATECHOLAMINE RELEASE FROM FETAL ADRENAL MEDULLARY CELLS IN CULTURE: EFFECT OF HORMONES. Cecilia Y. Cheung. Div, of Perinatal Biol, Loma Linda Univ, Sch. of Med, Loma Linda, CA 92350

In the ovine fetus, circulating catecholamines as well as the hormones angiotensin II (AII) and prolactin (PRL) play a major role in the maintenance of cardiovascular homeostasis. We have recently observed that an i.v. administration of oPRL into ovine fetuses can induce increases in arterial pressure and heart rate (C.Y. Cheung, R.A. Brace, Soc. Gyn. Invest. Abs. #193, 1982). These responses occurred even in the presence of ganglionic blockade, suggesting that humoral agents rather than neural reflexes were responsible for the changes. To investigate the factors involved in the regulation of catecholamine release from fetal adrenal medulla, and to determine whether the effects of prolactin can be mediated through release of adrenal catecholamines, the direct effects of oPRL, AII and cortisol on the release of dopamine (DA), epinephrine (EPI), and norepinephrine (NE) from fetal adrenal medullary cells in culture were studied. The adrenal glands from ovine fetuses of 125-135 days gestation were used. The medulla was dissected free of the cortex and the tissues dispersed using 0.05% collagenase. The dispersed cells were allowed to plate for 18 h with 10^5 cells per well in Kreb's Henseleit buffer containing 0.5% BSA at 39.5°C in a humidified atmosphere of 95% air and 5% CO_2. Following a 6 h preincubation, fresh medium alone or containing oPRL (40 ug/ml), AII (40 ug/ml), or cortisol (10^{-6}M) was added to the cells and the incubation was carried on for 24 h. The medium was sampled at 3, 6, 18, and 24 h for measurements of DA, EPI, and NE using a radioenzymatic assay. oPRL enhanced the release of DA, EPI, and NE at 6 h by 103%, 75%, and 217%, resp. AII stimulated the release of EPI and NE at 6 h by 51% and 100%, resp., while decreasing the release of DA at 3 h by 47%. Cortisol selectively increased the release of EPI at 6, 18, and 24 h by 119%, 222%, and 167% resp., but had little effect on the release of DA or NE. In other experiments, the adrenal cortical cells were dispersed separately and recombined with medullary cells. The amount of DA, EPI, and NE released into the medium from medullary cells at 2 h was 43 ± 15, 565 ± 24, and 869 ± 75 pg/ml resp. However, in medullary cells recombined with cortical cells, the release of catecholamines was significantly elevated to 253 ± 25, 1510 ± 97, and 10,298 ± 825 for DA, EPI, and NE resp. The elevation in catecholamine release was maximal at 6 h, and continued to be observed at 24 h. The results suggest 1) oPRL can stimulate fetal adrenal medulla to release DA, EPI, and NE which could cause major hemodynamic effects on the fetal circulation, 2) AII and cortisol can enhance the release of NE and/or EPI from fetal adrenal medulla, and 3) recombination of fetal adrenal medullary cells with cortical cells can potentiate the release of all 3 catecholamines.

EPILEPSY: PHYSIOLOGY II

266.1 NEONATAL ALTERATION OF CENTRAL NORADRENERGIC AXONS ABOLISHES INHERITED SPIKE WAVE ABSENCE SEIZURES IN THE MUTANT MOUSE TOTTERING. Jeffrey L. Noebels, Departments of Neuropathology, Harvard Medical School and Neuroscience, Childrens Hospital, Boston, Massachusetts.

Tottering mice (tg) show in adolescence stereotyped spike-wave discharges with behavioral absence seizures. The sole CNS cytopathology as yet uncovered is a selective overgrowth of locus coeruleus axons, raising NE levels 100-200% within most LC terminal fields (Levitt and Noebels, PNAS 78:4683, 1981). To evaluate the pathogenetic significance of LC hyperinnervation with regard to the neurologic expression of the tg gene, homozygous tg/tg littermates were injected subcutaneously with the neurotoxin 6OHDA (100mg/kg) or saline on the 1st and/or 2nd postnatal days. EEG recordings were obtained daily from ages 18days-15weeks. In the untreated adult tg/tg, 6-7/sec spike-wave discharges (1-10sec., 300-400mV) occur at a mean rate of 50/hr.

Neonatal injections of 6OHDA entirely prevented the later appearance of cortical discharges and behavioral absence seizures without altering the otherwise normal EEG rhythms of freely behaving mice. In 5/9 tg/tg's, the effect was absolute; histofluorescence studies at age 15 weeks revealed complete absence of NE axons in all cortical laminae, paralleled by 94-97% decreases in cortical NE levels (assayed by HPLC-EC) with respect to untreated mutants. In the remaining 4 mutants, spike-wave bursts were delayed by 4 weeks, and developed at greatly reduced rates (1-2/hr), duration (0.3-1.0sec), and amplitude (100-200mv). Fluorescence surveys in these mice revealed scattered LC axon patches in all neocortical layers, suggesting that the initial lesion was incomplete.

To determine whether LC denervation could acutely reverse the abnormal CNS synchronization once the neurological traits of the tg gene were fully expressed, unilateral stereotactic injections of 6OHDA were made into the LC of 3 adult tg/tg mice. Initially (48hrs), discharge rate and duration increased sevenfold; over the subsequent 10 weeks, the rate decreased 82% (mean 12 vs 66 bursts/hr); 75% of the decrease ocurred in the first week.

These findings relate the inherited expression of abnormal synchronization and absence seizures to a gene-linked error within a single neuromodulatory cell group, the locus coeruleus. The data indicate that the clinical disease can be arrested prior to its natural phenotypic expression, and that there is probably no critical stage in development which irreversibly commits the affected neurons to abnormal firing patterns. Supported by the Klingenstein Foundation.

266.2 THE EFFECT OF PHENYTOIN ON POST-TETANIC POTENTIATION AT THE FROG NEUROMUSCULAR JUNCTION. M.E. Selzer, G. David*, and Y. Yaari*. Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 and Department of Physiology, Hebrew University-Hadassah Medical School, P.O.Box 1172, Jerusalem, Israel 91010.

Phenytoin (diphenylhydantoin, DPH) is known to reduce post-tetanic potentiation of spinal reflexes. The mechanism for this effect is not known, but it has been suggested that it depends on a blockage of invasion of the action potential into nerve terminals at high frequencies of stimulation.

We studied the effects of DPH on the frequency dependent activity of the endplate potential at frog (R. ridibunda) sartorius and cutaneous pectoris neuromuscular junctions under conditions of low quantal release (0.4-1 mM Ca++, 4 mM Mg++). Both surface electrodes, which sampled the simultaneous endplate currents of several endplates, and intracellular microelectrodes were used. In both types of experiments, bathing the preparation for one hour in 0.2-0.4 mM DPH produced a dramatic reduction (up to 100%) in tetanic potentiation (TP) during a 30 sec - 30 Hz tetanus. Post-tetanic potentiation (PTP) was also greatly reduced, especially the early phase (augmentation). By contrast, facilitation was unaffected by DPH.

With intracellular recordings, it was found that the reduction of PTP (up to 100% for the early phase and 50% for the late phase) could occur even when there were no failures of endplate potentials during the tetanus. Thus, there were no failures of the presynaptic action potential.

Similar effects were seen at lower doses of DPH (.03-.1 mM) if the muscles were exposed to the drug for several hours.

It is concluded that at the frog neuromuscular junction: 1) DPH greatly reduces TP and PTP by a mechanism which does not require failure of invasion of the presynaptic terminal by the action potential during the tetanus; 2) the efects are specific for TP and PTP because under the present conditions, neither facilitation nor the mean quantal content of unpotentiated endplate potentials was reduced; and 3) the large doses of DPH often required to produce significant effects in acute experiments may reflect its slow onset of action.

USPHS #F06 TW00697, the U.S.-Israel BSF and the Meller Endowment Fund.

266.3 PAROXYSMAL DEPOLARIZATION AND HYPERSYNCHRONICITY IN VIRAL INFECTED GANGLIA, by Jacob Zabara, Physiology/Biophysics, Temple University, Philadelphia, PA. 19140.

Hypersynchronicity and paroxysmal depolarization (PD) are observed in viral infected ganglia and related to neuronal discharge synchronization in epileptic seizures. The superior cervical ganglion, infected with pseudorabies virus, is investigated as an experimental model of seizure discharge as occurs in focal or generalized epilepsy. In this preparation, the development of seizure-like discharges can be observed in a relatively isolated synaptic region where electrophysiological techniques can be used to monitor the underlying processes. The superior cervical ganglion is infected by intraocular viral (pseudorabies) inoculation of the right eye of rats. Recordings are performed either in situ or after excision of the ganglion which is suspended on Ag-Ag Cl electrodes in a humidified chamber containing a modified Ringer's solution and equilibrated with 95% O_2, 5% CO_2. Recordings are performed of evoked potentials (by rectangular pulses applied to the presynaptic nerve), and of potentials generated by viral replication, with d.c. amplifiers and oscilloscope. PD's begin as a consequence of viral replication in the postsynaptic cell bodies. These PD's have a characteristic rise time of approximately 50 msec., a duration of approximately 200 msec. and occur at a frequency of approximately .5Hz. Spike bursts are superimposed on the rising phase and peak of the PD's. Perfusing the superior cervical ganglion with acetylcholine decreases the PD interval time and amplitude but does not disrupt the rising or falling phases of the PD's. An analysis of time delays indicates that there is no single pacemaker cell or group of pacemaker cells from which the PD originates. These observations support the possibility that the PD represents a field potential which acts externally (i.e., ephaptically) in a relatively large population of cells to synchronize spike discharges in the cell population. This synchronization is related to viral replication since as the infection proceeds, increasing waves of oscillatory depolarization occupy the population of neurons producing field bursts of increasing amplitude and duration. A slow IPSP appears to be associated with the PD's in early stages of infection. The slow IPSP progressively diminishes with time until a later stage of infection when oscillatory waves of depolarization make their apperance. It is possible that inhibitory neurons within the ganglion (SIF cells) are involved in the increasing synchronization of spike discharges. Thus the PD's and hypersynchronicity could have their origin in viral replication within inhibitory cell bodies.

Supported in part by a T.U.R.I.F. grant.

266.4 ACTION OF TUNGSTIC ACID GEL ON CAT LUMBAR MOTONEURONS. W. Spain*, P. C. Schwindt* and W. E. Crill. Depts. Physiology & Biophysics and Medicine, University of Washington; Veterans Administration Medical Center, General Medical Research (151), Seattle, WA 98108.

Injection of tungstic acid gel (but not pH adjusted saline) into the cat lumbar ventral horn results in spontaneous, epileptiform bursts of action potentials in motoneurons surrounding the injection site. The abnormal motoneuron activity consists of waves of repetitive grouped discharges, or bursts, of action potentials. Each burst is composed of 3-6 action potentials where action potentials subsequent to the first one are triggered from the delayed depolarization of the preceding action potential. The same kind of multi-action-potential, high frequency bursts can be triggered by brief intracellular injected current pulses which evoke single action potentials in normal motoneurons. Rhythmic, repetitive bursts of the same type are evoked by long-lasting injected current pulses. Thus, altered neuronal membrane properties are responsible for the bursting activity. The character of the bursts observed after tungstic acid application differ markedly from those seen during strychnine- or penicillin-induced spinal seizures and is likely to result from different cellular mechanisms. It is hypothesized that the alteration of fast-activated ionic conductances and/or dendritic properties underly the tungstic acid-induced bursting as opposed to the alteration of slow ionic conductances which are involved in penicillin-induced bursting.

Supported by the Veterans Administration and NIH Grant NS 16792.

266.5 NEURONAL ACTIVITY IN AREAS OF CHRONIC CORTICAL INJURY. J.W. Lighthall* and D.A. Prince (SPON: M. Scher). Dept. of Neurology, Stanford Univ. Sch. of Medicine, Stanford, CA 94305.

We used in vitro techniques (37°C) to study neurons in guinea pig sensory motor neocortical slices (500 μm) obtained from chronic epileptogenic freeze lesions made 1-3 wks earlier. Intracellular recordings were obtained from 42 neurons in cortical laminae located directly beneath the site of the freeze lesion. The properties of 18 neurons examined in detail were as follows: V_m = 69.8 ± 11.8 mV; spike amp = 8.10 ± 13.3 mV; and time constant = 11.5 ± 3.8 msec. Following orthodromic (intracortical or subcortical) stimulation, 16 of these neurons generated fixed short latency bursts of action potentials superimposed on long duration (62.5 ± 15.2 msec) graded multiple component depolarizations. Three of 18 neurons exhibited variable (75-150 msec) latency bursts of 1-6 action potentials. The duration and amplitude of both fixed and variable latency depolarizations, and the number of spikes varied with stimulus intensity and frequency. Both types of depolarizing events resembled PSPs in their response to changes in V_m. Similar depolarizations and burst responses could not be evoked by intracellular current injection, nor were they observed in 10 layer IV neurons recorded in normal guinea pig neocortex. Four neurons exhibiting a short latency graded burst response were intracellularly labelled with HRP. These consisted of 3 layer IV medium-sized spiny pyramidal neurons and 1 layer III multipolar spiny stellate neuron. Dendrites of these cells which subtended the lesion site showed no gross morphological abnormalities.

These preliminary data indicate that neurons in the area of a chronic freeze lesion possess unusual orthodromic response characteristics. Both short and variable latency burst responses were unlike the all-or-none intrinsic bursts observed in some normal layer IV neurons (Connors et al., J. Neurophysiol. 48:1302, 1982). The long variable latency depolarizations and bursts had properties similar to those of neurons in chronic epileptogenic cortex of man (Prince and Wong, Brain Res. 210:323, 1981) and depolarizing shifts in neurons of penicillin-treated neocortical slices (Gutnick et al., J. Neurophysiol. 48:1321, 1982). Our data suggest that the burst responses in chronic freeze lesions are the result of modified synaptic impingement; alterations in intrinsic membrane properties may also be present, but have not as yet been identified. The role of such cellular behavior in the generation of epileptiform events in the chronic focus in vivo is unclear.

Supported by NIH grant NS 12151 from the NINCDS.

266.6 INTRACELLULAR RECORDINGS FROM HUMAN EPILEPTIC CORTEX: *IN VITRO* MAINTENANCE OF SPONTANEOUS RHYTHMIC ACTIVITY. P.A. Schwartzkroin and W.D. Knowles, Dept. Neurological Surgery, Univ. Washington, Seattle, WA 98195

The *in vitro* slice preparation has made it possible to obtain intracellular recordings from neurons in cortical tissue removed from a suspected epileptic focus in human epilepsy patients. Our earlier studies on slices from lateral temporal neocortex, however, yielded little convincing evidence that epileptiform activity could be maintained *in vitro* (Ann Neurol 13:249-257, 1983). We now report results of more recent experiments in which unusual (epileptiform?) activity has been observed.

Human tissue was obtained during neurosurgical procedures to remove focal epileptic regions of temporal lobe in patients with medically-intractable seizures. Cortical biopsy samples from both lateral and mesial temporal lobe were cut into slices 700-800 μm thick, and studied electrophysiologically. Spontaneous cellular activity was present in both lateral and mesial tissue samples, but there were no spontaneous field potential events suggestive of epileptic 'spikes'. Stimulation in the white matter region of the slice evoked synaptic inputs in cortical neurons consisting of both EPSP and IPSP components. Average cell resting potential, action potential amplitude, and input resistance were similar for cells in lateral and mesial cortex.

The most striking activities recorded in these experiments were spontaneous, rhythmic postsynaptic potentials. These PSPs, consisting of both depolarizing and hyperpolarizing components, were seen most often in the tissue from mesial temporal lobe. The depolarizing component of the PSP was sometimes sufficiently large to trigger action potentials. Stimulus-evoked and spontaneous PSPs had a similar appearance, with similar reversal potentials for their respective hyperpolarizing components. Spontaneous PSP frequency was not dependent on cell resting potential, but PSPs were blocked when TTX was focally applied near the recording electrode. Simultaneous intracellular recordings from two cells were obtained from several tissue samples in which this rhythmic activity was apparent; in all cases, these spontaneous events occurred synchronously, or with a close phase-locked relationship, over a wide area of a slice.

The mechanism(s) underlying these events is unclear. There may be some pacemaker region in the tissue, and/or the synchronous activity may be the result of unstable circuit interactions. It appears that the requisite cells and circuitry are more likely to be contained in thicker slices, and in tissue from mesial temporal cortex than in thinner and/or lateral cortex slices. These spontaneous, rhythmic, synchronous PSPs may reflect the epileptogenic nature of the tissue from human foci.

(Supported by grants NS 00413, NS 17111, and BNS 7915115).

266.7 MECHANISMS IN HIPPOCAMPAL CA3 REGION WHICH PROTECT AGAINST SPREADING DEPRESSION. M.M. Haglund and P.A. Schwartzkroin Dept. of Neurological Surgery, University of Washington, Seattle, WA 98195.

Using the slice preparation of the developing rabbit hippocampus, we have studied spontaneous spreading depression (SD) episodes that occur at highest frequency at 8-13 days postnatal. These spontaneous SD events are primarily limited to the CA1 region. The present investigations were aimed at elucidating the "protective" mechanisms among the CA3 cells which prevent the spread of depolarization into this region.

Simultaneous intracellular recordings from the CA1 and CA3 regions were obtained to analyze the sensitivity of the two regions to spontaneous SD episodes and their thresholds to incoming SD. In order to control the onset of SD, 2 M KCl was applied with pressure ejection (30 psi) from a microelectrode (2-5 µm tip, 50-150 msec) located in stratum pyramidale either 100 µm away from an intracellular electrode or midway between the two intracellular electrodes. The SD episodes elicited with this technique had similar chracteristics to those SD episodes which occurred spontaneously (see Neurosci. Abstr. 8:1017,1982). The SD episodes induced by KCl application in the CA1 region led to two types of activity in the CA3 cells: 1) The CA3 cell would increase its spontaneous firing rate, then hyperpolarize 5-10 mV, and finally return to resting membrane potential level as the CA1 cell repolarized; or 2) Ictal activity began in the CA3 region 5-20 seconds after the onset of a SD episode in the CA1 region. The CA3 seizure consisted of a 10-20 mV depolarization which developed into rhythmic discharges. Upon repolarization of the CA1 cell, the ictal activity in the CA3 region ceased, and synchronous afterdischarge bursts were observed.

That the CA3 region has a higher threshold to the SD was confirmed in experiments in which potassium applications were made between the two cell regions. In most cases, the potassium application triggered SD in the CA1 region, while the CA3 cells either hyperpolarized or generated ictal activity. Potassium pulse durations of 50-100 msec elicited SD in the CA1 region, whereas durations of 200-300 msec (through the same electrode) were needed to trigger a SD episode in the CA3 region. Frequency of SD episodes in both the CA1 and CA3 regions was increased when the $[Cl^-]_o$ of the bathing medium was lowered from 120 to 20 mM; the spontaneous and potassium-elicited SD events were eliminated by blocking synaptic transmission ($[Ca^{++}]_o$ to 0.5 mM and $[Mg^{++}]_o$ to 8.0 mM). These results indicate that SD episodes are dependent upon synaptic transmission and suggest a critical role for local inhibitory mechanisms. The IPSPs in CA3 cells, which are potent much earlier in development than CA1 IPSPs, may account for the CA3 resistance to SD.

(Supported by NS 15317, NS 00413, and NS 17111)

266.8 KAINATE LESIONED HIPPOCAMPI BECOME EPILEPTOGENIC. J.E. Franck* and P.A. Schwartzkroin (SPON: M. Anderson) Department of Neurological Surgery, Univ. Washington, Seattle, WA 98195.

It is well known that kainic acid creates an hippocampal lesion remarkably like that seen in Temporal Lobe Epilepsy. In clinical material, hippocampal necrosis appears as a selective loss of discrete pyramidal cell subfields; other subfields, as well as the dentate granule cells, are left apparently viable. The kainate model provides an opportunity to study whether such a selective pattern of cell death, and the intrinsic reorganization that occurs in remaining neurons, is a benign by-product of repeated seizures or if it also may contribute to epileptogenesis.

The CA3-CA4 region of the rat hippocampus was destroyed by unilateral, intraventricular kainate injection (0.5ug in 1ul saline, pH=7.4). Three weeks later pyramidal cells in the CA1 region were studied in the in vitro slice preparation.

Subfield CA1, in lesioned slices, generated both multiple population spikes and synchronous afterdischarge activity to orthodromic stimulation. When individual neurons from these slices were examined, there was a considerable attenuation of the afterhyperpolarization (AHP) that normally follows current induced repetitive firing. Often this was replaced by a depolarizing afterpotential (DAP). Prolonged DAPs were also seen following spontaneous activity. These DAPs often induced additional spike firing which, in some cells, progressed to seizure activity riding on a long depolarizing envelope.

There were numerous, apparently healthy, interneurons in slices with afterdischarge activity. However, the IPSP normally seen in pyramidal cells following antidromic stimulation was difficult to elicit, and was sometimes replaced by multiple EPSPs or additional orthodromic spikes.

The AHP and DAP observed in pyramidal cells normally control the occurrence and frequency of burst firing. The prominence of the DAP in cells from lesioned hippocampi may be a mechanism for alterations in excitability in these neurons. In addition, while interneurons are present, the relative absence of IPSPs in pyramidal cells suggests that pyramidal cell-interneuron connectivity has been altered. These data indicate that, at this survival period, lesioned hippocampi have become progressively epileptogenic and that this response is due to a loss of both intrinsic damping mechanisms and extrinsic inhibitory control.

(Supported by grants NS00413, NS07144 and NS17111)

266.9 HIPPOCAMPAL ELECTROPHYSIOLOGY AFTER KAINIC ACID TREATMENT: A CHRONIC MODEL OF FOCAL EPILEPSY. B. Lancaster,* H.V. Wheal,* and T.J. Ashwood.* (SPON: P.R. Adams). Dept. of Neurophysiology, University of Southampton, Southampton SO9 3TU, U.K.

The neurotoxin kainic acid (KA) can produce acute seizure activity, and intracerebral KA produces lesions which resemble the pathology often associated with temporal lobe epilepsy. However, there are few studies on the chronic epileptogenic effects of KA-induced lesions. This report describes some electrophysiological changes seen in CA1 pyramidal neurones in the KA-lesioned hippocampus. Unilateral lesions of the CA3/CA4 area were made by stereotaxic injection of KA into one lateral ventricle of an anaesthetized rat (Lancaster & Wheal, J. Comp. Neurol. 211:105). At post-operative day seven, transverse slices of the hippocampus were prepared. Control slices were obtained from the contralateral, unlesioned hippocampus or from untreated animals. The potential KA model was evaluated by comparison with preparations in which GABA-mediated inhibition was blocked by bicuculline.

Intracellular recordings from CA1 pyramidal neurones showed that synaptic stimulation normally evoked only a single action potential (AP), followed by a biphasic afterhyperpolarization (AHP). In common with previous observations (Nicoll & Alger, Science 212:957) the first phase of the AHP, and also the recurrent IPSP evoked by stimulation of the alveus were blocked by bicuculline, suggesting that they are GABA-mediated. This loss of inhibition was accompanied by synaptically evoked bursting activity. In the KA-lesioned hippocampus, CA1 pyramidal cells displayed values for resting potential, input resistance and AP amplitude which were indistinguishable from controls; however, synaptic activation evoked burst responses in 55% of neurones studied. All of these cells showed changes in the characteristic orthodromic AHP. The first phase was either markedly reduced or more usually absent (80%); and the second phase was also significantly reduced. The recurrently evoked IPSP was reduced or absent in comparison to control recordings.

KA treatment is known to induce spontaneous, recurrent seizure activity (Pisa et al., Brain Res. 200:481). In addition, it produces a characteristic pathology and a reduction in GABA-mediated inhibition associated with burst discharges. The results suggest that the KA-lesioned hippocampus may be a suitable chronic model of epilepsy.

Supported by a Wellcome Trust grant (HVW) and travel grant (BL), and the MRC (UK). HVW is a Wellcome Trust Senior Lecturer. Present address of BL; Dept. of Neurobiology & Behavior, SUNY at Stony Brook, New York 11794.

266.10 ELECTROPHYSIOLOGY OF KAINIC ACID (KA) INDUCED EPILEPTIFORM ACTIVITY IN THE RAT HIPPOCAMPAL SLICE. R.S. Fisher* and F.E. Alger, Dept. Physiol., Univ. Maryland Sch. Med., Baltimore, MD 21201.

Depression of GABA-mediated IPSPs has been proposed to be a crucial factor in the onset of epileptiform activity in most models of epilepsy. To test this idea, we induced epileptiform activity by bath application of 0.3-1 µM KA in the rat hippocampal slice. Repetitive field potential firing, spontaneous or evoked, occurred during exposure to KA. Intracellular records from 30 CA1 pyramidal cells during changes between control saline and saline containing 1 µM KA indicated that KA depolarized cells an average of 5 mV and caused a 15% decrease in input resistance. In several cells the tonic effects were preceded by a transient phase of sporadic, spontaneous depolarizations of 2-10 mV which lasted 50 - 200 ms. These small depolarizations, which could trigger action potentials, were blocked by membrane hyperpolarization. Action potentials and current-induced burst afterhyperpolarizations did not change significantly.

The major effect of 1 µM KA was a reversible depression of both fast, GABA-mediated IPSPs and slow, potassium (K) dependent IPSPs. These IPSPs were reduced, respectively, to 20% and 31% (corrected for resistance change) of control values. IPSP depression correlated closely with the onset of burst potential firing in reponse to synaptic stimulation. KA did not increase EPSP amplitudes. Similar observations were made on 6 CA3 cells, although these cells were affected by lower doses of KA.

The mechanism of IPSP depression was studied by using KCl filled electrodes to invert small spontaneous IPSPs into depolarizing potentials. In 9 CA1 cells the rate and amplitude of spontaneous IPSPs transiently increased, but then decreased in conjunction with evoked IPSP depression. Possible KA effects on postsynaptic GABA responses were investigated with GABA iontophoresis. In KA hyperpolarizing GABA responses were variably decreased but these changes could not account for KA induced IPSP depression measured concurrently.

KA-induced alterations in extracellular K were assessed in 6 slices with ion-sensitive microelectrodes and in 2 slices with glial cell penetrations. The increases of approximately 0.5 mM in extracellular K could not explain the effects of KA.

We conclude that KA induces epileptiform activity in hippocampus principally by a presynaptic block of IPSP pathways. Supported by NIH Grant NS17539 and a McKnight award (E.E.A.) and by TIDA 5K07NS00697 (R.S.F.).

266.11 CL 218, 872 BLOCKS KAINIC ACID-INDUCED CONVULSIONS AND NEUROPATHOLOGY, J.F. Bates*, L.J. Standish* and T.W. Hall*. (SPON: A. Trehub).

The convulsive behavior and neuropathology associated with human status epilepticus can be induced in laboratory rats with intraperitoneal injections of kainic acid (KA), a rigid analogue of glutamic acid with neuroexcitatory properties. A benzodiazepine, diazepam (DZ), is the drug of choice in treating status epilepticus. DZ has been reported to prevent KA-induced convulsions in laboratory rats at a dose of 20 mg/kg i.p., as well as reducing the extensive limbic system neuropathology that accompanies KA-induced status epilepticus. Triazolopyridazines (TPZs) are a novel class of drugs with potent anti-anxiety effects in experimental animals and selective affinity for Type I benzodiazepine receptors. We compared the anti-epileptic effects of CL 218,872, a potent TPZ, and DZ with respect to behavioral and neuropathological protection against the epileptogenic action of KA.

Rats were infected intraperitoneally with KA alone (12 mg/kg), or pretreated with either CL 218,872 (1.7, 25, 50, or 150 mg/kg) or DZ (20 mg/kg) prior to KA (12 mg/kg). Behavior was scored every 10 minutes over a 6 hour period, using a scale of 0-15 (normal to grand mal convulsion). Following a 7 day survival period, animals were perfused intracardially with formalin. Tissue was subsequently sectioned and stained using a modified Fink-Heimer silver degeneration procedure. Coronal 40 μ sections were analyzed by light microscopy for the presence of degenerating cells and necrosis.

KA treatment resulted in severe (generalized tonic-clonic) convulsions and extensive brain damage in limbic system structures (hippocampus, amygdala, lateral septum, midline, medial and lateral thalamic nuclei and limbic cortex) and several neocortical areas. Pretreatment with CL 218,872 produced dose-dependent effects. Doses of 50 and 150 mg/kg significantly reduced the severity of KA-induced convulsions, as well as reducing, or preventing, neuropathology. In contrast to previous reports, DZ pretreatment did not significantly reduce convulsion severity, nor did it protect against KA-induced neuropathology.

CL 218,872 is a potent anti-epileptic drug with respect to both convulsions and neuropathology associated with KA-induced status epilepticus. In addition, this TPZ was more effective than DZ. These results suggest that CL 218,872 in particular, and TPZ's in general, may be of clinical value as anti-epileptic agents in the treatment of status epilepticus.

266.12 PROPERTIES OF RECURRENT EXCITATION IN THE CA3 REGION OF THE HIPPOCAMPUS. R. Miles* and R.K.S. Wong (SPON: J.J. Stewart). Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

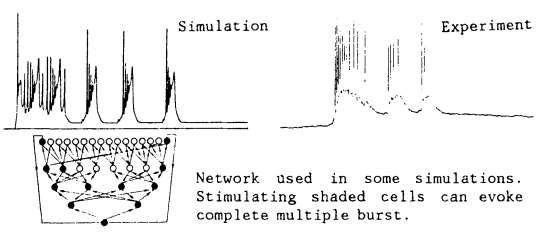
Local circuit interactions are of great importance in modulating neuronal activity. In the hippocampus the existence of recurrent inhibition is well established. However although local excitatory connections are thought to exist much less is known about them. In this study we have attempted to demonstrate and define the characteristics of recurrent excitatory synapses between pyramidal cells in the CA3 region of guinea pig hippocampal slices.

In order to test for recurrent excitation between CA3 neurones we stimulated in the stratum oriens or radiatum of the CA1 region where axonal collaterals of CA3 cells are known to project. A proportion of CA3 neurones were antidromically activated while in most other cells an epsp followed by an ipsp was recorded. The ipsp was blocked in the presence of 10^{-4} M picrotoxin leaving a series of epsp's which often initiated an epileptiform burst. Increasing Mg to 8 mM allowed a pure epsp to be examined in the absence of synchronized activity. This synaptic potential could result from the stimulation of cholinergic septal fibres or commissural fibres from the contralateral hippocampus. However it could still be evoked in the presence of 10^{-6} M atropine and in slices prepared from guinea pigs where commissural fibres had been allowed to degenerate following a midline lesion. Based on its latency and frequency following ability and on the fact that the epsp could still be evoked in 14 mM Mg when di-synaptic recurrent inhibition was blocked we conclude that this synaptic potential results from monosynaptic recurrent connections between CA3 neurones.

With low intensity stimulation 'minimal' synaptic events of varying amplitude interspersed with a proportion of transmission failures could be evoked. These synaptic potentials were comparatively slow with mean time to peak of 8.9 msec and mean duration at half amplitude of 32.2 msec. Their mean amplitude in the presence of 8 mM Mg was 0.9 mV (n=17). The application of paired stimuli separated by times from 20 to 1000 msec revealed a potentiation of 57% at 20 msec which declined exponentially with time constant 92 msec (n=8). The determination of these properties of recurrent excitatory synapses provides a basis for the exploration of their role in local integration processes in the CA3 region of the hippocampus. (Supported by NIH grant NS18464).

266.13 SIMULATION OF IN VITRO EPILEPTIFORM MULTIPLE BURSTS, R.D. Traub, W. D. Knowles, R. Miles, R.K.S. Wong and R. Linsker*. IBM Watson Research Center, Yorktown Heights, NY 10598 and UTMB, Galveston, TX 77550.

When the hippocampal slice is bathed in convulsant agents (e.g. penicillin), synchronized neuronal bursting occurs. We have shown how chemical excitatory synaptic interconnections can lead to a long-latency single population burst (Traub and Wong, Science 216 (1982) 745-747). In the presence of the GABA-blocker picrotoxin (0.1mM), more complex spontaneous events occur: multiple bursts rather than single bursts. All cells observed participate in each event. Multiple bursts consist of a sustained (about 150 ms) primary burst followed by a series of 1 to 5 or more afterdischarges occurring every 50-65 ms. Multiple bursts are synchronized. As for synchronized single bursts, both the primary burst and the afterdischarges of multiple bursts are synaptically elicited (R. Miles and R.K.S. Wong, Soc. Neurosci. Abs. 8 (1982) 910). Synchronized activity can be partially entrained and the rhythm reset by intracellular stimulation of some, but not all, CA2-CA3 cells. In simulation studies, we used a single compartment model for each neuron, because the soma and apical dendrites are synchronized during multiple bursts. We made parameter changes in the model neuron that were forced by the experimental data. These allowed for shortening the interburst interval, slowing the kinetics of burst termination, changing the burst afterhyperpolarization to a depolarization, and preventing depolarization block. We made networks of model neurons connected by excitatory synapses. Synaptic inhibition was not included, because of its presumed blockade by picrotoxin. Randomly connected networks lead to realistic multiple bursts in individual cells, but without synchronization between cells. Tree-like networks containing loops generated, after stimulation of some single cells, synchronized multiple bursts similar to those observed experimentally. Decreasing synaptic strength produced, as required, a decreased number of afterdischarges.



Network used in some simulations. Stimulating shaded cells can evoke complete multiple burst.

266.14 CHANGES IN BRAIN WATER CONTENT AND PERMEABILITY OF THE BLOOD-BRAIN BARRIER (BBB) IN CONVULSIVE SEIZURES. R. Cahn*, T. Kuroiwa*, C. Nitsch*, W. D. Lust and I. Klatzo. Lab. of Neuropathology and Neuroanatomical Sciences and Lab. of Neurochemistry, National Institutes of Health, Bethesda, MD 20205.

The purpose of this study was to evaluate the effect of short epileptiform seizures on brain water content and behavior of the BBB. The convulsive seizures were induced by intravenous injection of 40-50 mg/kg of pentylenetetrazole (PTZ). The behavior of the BBB was assessed with 2% Evans Blue (EB) tracer (0.5 ml intraven.). Brain water content was evaluated by modified specific gravity (SG) measurements; also, osmolarity and levels of lactate were assessed in the plasma. Two groups of animals: A) awake, non-paralyzed, spontaneously breathing, and B) anestnesized, paralyzed and artificially ventilated, were subjected to PTZ seizures lasting usually between 10-20 minutes and were sacrificed at various time intervals up to 6 hours following induction of the seizures.

Both groups of animals showed circumscribed areas of EB extravasation, most common in hypothalamus, cerebellum, thalamus and pallidum. In the non-paralyzed group there was a significant increase in SG values, more pronounced in EB-negative than in EB-positive regions, which reached its peak at 30 minutes and returned to normal after 3-6 hours. The increase in SG, indicating dehydration of the brain, coincided with a marked increase in blood osmolarity, which showed a sharp increase already 5 minutes after the onset of seizures and then gradually returned to normal, approximately 4 hours after convulsions. The plasma lactate changes in non-paralyzed animals parallel those concerning osmolarity with the peak at 5 minutes and return to normal at 4 hours.

In paralyzed, artificially ventilated animals the PTZ seizures produced similar changes in BBB, but no changes in osmolarity or plasma lactate. The SG values in EB-negative areas were normal, whereas those in EB-stained regions revealed significantly lower levels at 6 hours after the seizures.

Our investigations indicate that the epileptic seizures associated with strong tonic-clonic convulsions induce a marked elevation of blood osmolarity, presumably mainly due to a rapid increase in lactate and this leads to a significant transient decrease in water content of the brain. The opening of the barrier associated with extravasation of serum proteins leads to edematous water increment in areas with barrier damage.

267.1 A COMPARISON OF VISUAL CALLOSAL CONNECTIONS IN NORMAL, NEONATALLY BLINDED AND CONGENITALLY ANOPHTHALMIC MICE. R.W. Rhoades, R.D. Mooney, G. Yuen* and S.E. Fish. Dept. of Anatomy, UMDNJ-NJSOM & RMS, Piscataway, NJ 08854 and Neurobiology Program, N.E.O.U.C.O.M., Rootstown, OH 44272.

To assess the consequences of prenatal versus postnatal eye "removal" upon the development of visual callosal connections, the organization of this pathway was examined using HRP transport in normal adult C57BL and DBA mice, adult C57BL mice enucleated bilaterally within 12 hr of birth and adult ZRDCT-an congenitally anophthalmic mice.

In normal animals labelled callosal neurons and anterograde transport indicative of callosal terminals were located primarily at the borders of area 17. In the 17-18a border area labelled cells were located in primarily layers II, III and V. Anterograde transport was dense in layers V and VI, light in lamina IV and moderate in layers I-III. In addition, moderate anterograde labelling and a few retrogradely labelled cells were visible in lamina VI of the medial striate cortex.

In animals bilaterally enucleated on the day of birth and examined as adults the callosal projection was markedly abnormal. Both callosal cells and anterograde labelling had the same laminar distribution as in the normals, but they extended well into the medial part of area 17.

In adult, anophthalmic mice callosal cells and terminals were also visible medially, particularly in the rostral part of area 17. Here, this labelling took the form of discrete patches, approximately 275μm in width which were separated by regions of approximately the same size that were largely free of labelled callosal cells or terminals. In serial sections, these patches formed bands which paralleled the 17-18a border. The laminar distribution of labelled cells and fibers at the 17-18a border in these animals matched that in the normals. In the more medial patches, however, labelled cells were restricted primarily to layers II and III.

The above results suggested that the presence of the retinae prenatally may have some influence upon the development of visual callosal connectivity. Surprisingly, however, the distributions of callosal cells in normal and anophthalmic animals examined on postnatal day 3 were identical and extended uniformly over the mediolateral extent of the posterior neocortex.

Supported by EY03546, EY04710, BNS8004601, DE06528, The March of Dimes and the UMDNJ Foundation (RWR) and NS18369 (SEF).

267.2 COGENERATION OF SUBPLATE AND MARGINAL ZONE CELLS IN THE CAT'S PRIMARY VISUAL CORTEX. M.B. Luskin and C.J. Shatz. Department of Neurobiology, Stanford, CA 94305.

During the development of the cerebral cortex, the Retzius-Cajal cells are among the earliest generated, and come to lie within the marginal zone (MZ)--future layer 1. These cells are numerous throughout embryonic life, but only a few survive into adulthood (Edmunds, S.M. and Parnavelas, J.G., J. Neurocytol., 11:427, 1982). We reported (Neurosci. Abst., 8:3, 1982) that another sparse population of early generated cells was present below layer 6 embedded in the white matter. These cells are generated throughout the period between embryonic day 24 (E24) and E30 in the cat (gestation is 65 days) that largely precedes the neurogenesis of the cellular layers of the cortex. We wondered if, like the Retzius-Cajal cells, these white matter cells also might be more abundant during embryogenesis. Therefore intrauterine injections of ^{3}H-thymidine were made into 24 fetuses between E24 and E30, and autoradiographs of the regions of the occipital were examined at progressively later developmental ages from E34 through birth.

At every age a bistratified labeling pattern was found. Radioactively labeled cells were confined to one zone immediately deep to the cortical plate (CP), and another one within the MZ. In contrast to the extremely sparse cell labeling found in adults, extensive labeling of both zones was seen at every embryonic age. The deep zone is the subplate, a loosely packed layer of large darkly staining cells underlying the radially aligned densely packed cells of the CP. In cresyl violet stained sections, this zone appears by E30, the earliest time that the CP itself can be identified, and persists at least until birth.

This bistratified labeling pattern indicates that cells of the subplate and MZ are cogenerated in time. If so, then do the two sets of cells originate as a single population? To address this question, we injected a series of fetuses at E24 (a time well before genesis of the CP) and examined them at E30, E34, E41, E57 and adulthood. As before, the characteristic bistratified pattern was present in all animals aged E34 and older. However, at E30 the distribution of labeled cells was not bistratified. Instead, a single zone of labeling extended from the pial surface inward to the outer border of the ventricular zone.

These findings show that the subplate and MZ cells are generated concurrently as a single population, and later are split apart by the subsequent accretion of cells forming the CP. Thus, both sets of early generated cells may provide a framework for the development of the cortical plate that largely disappears when the process is complete.

(Supported by NIH grants EY02585 to C.J.S. and NS07158 to M.B.L.)

267.3 MONOCULAR DEPRIVATION EFFECTS IN THE VISUAL SYSTEM OF SQUIRREL MONKEY. M. Tigges, A. E. Hendrickson and J. Tigges . Yerkes Regional Primate Research Center, and Depts. of Anatomy and Ophthalmology, Emory Univ., Atlanta, GA 30322, and Dept. of Ophthalmology, Univ. of Washington, Seattle, WA 98195.

Four squirrel monkeys had their right eyelid sutured at or shortly after birth and survived from 9 to 40 months of age. Their brains were studied with various anatomical techniques, including the 2DG method, the transneuronal 3H amino acid and retrograde HRP transport techniques, and the cytochrome oxidase (CO) and Nissl stains. In all 4 monkeys, neurons in the deprived laminae of the LGN were considerably smaller in size compared to the neurons in the undeprived laminae. The retrograde transport of HRP after injections of this tracer into area 17 revealed that the cortical projections originating from the deprived laminae were still present since HRP filled neurons in the LGN extended in the typical shape of a wedge throughout all LGN laminae. The striate cortex proved remarkably resistant to extraneous manipulation. No changes in cell size and cell density were found in layer IVC of area 17 in Nissl stained sections. Also, CO staining resulted in a "puff" pattern in layers II-III, undistinguishable from that found in normal animals. Layers IVC and IVA were uniformly stained and, thus, were identical with the normal pattern. Transneuronal transport of monocularly injected 3H amino acid did reveal changes in the deprived striate cortex; these include the occurrence of irregularly spaced dense "patches" of silver grains over layer IVC in the ipsilateral striate cortex in the calcarine fissure. Compared to undeprived monkeys, layer VI in the deprived monkey contained only grains at background levels. Ipsilateral layer IVA in deprived animals was indistinguishable from that in normal monkeys. In layer IVC of contralateral area 17, a very few faint "patches" superimposed on a background of uniform labeling were found. Long stretches of contralateral layer IVA were unlabeled, except for a short strip in the anterior calcarine fissure, adjacent to the 17/18 border. After monocular visual stimulation of the deprived eye, 2DG labeling showed crisp patches of dense label in layer IVC superimposed on a uniformly high background; these were present in both hemispheres. These modest changes after monocular deprivation in squirrel monkey contrast with the dramatic effects on ocular dominance columns reported in infant macaca monkeys.

(Supported by NIH grants EY-00683, EY-01208 and RR-00165.)

267.4 THE PATTERN OF OCULAR DOMINANCE COLUMNS IN AREAS 17 AND 18 OF NORMAL AND VISUALLY DEPRIVED CATS AS REVEALED IN TANGENTIAL SECTIONS OF THE UNFOLDED CORTEX. P.A. Anderson*, J. Olavarria* and R.C. Van Sluyters. School of Optometry and Neurobiology Group, University of California, Berkeley, CA 94720.

We have used a procedure for unfolding and flattening the cortex (see Olavarria and Van Sluyters, this meeting), in combination with the transneuronal transport of horseradish peroxidase-wheat germ agglutinin conjugate (WGA-HRP), to study the overall pattern of ocular dominance (OD) columns in tangential sections through the full extent of areas 17 and 18 of the cat. In contrast to the autoradiographic method, use of WGA-HRP as a tracer allowed us to see the cortical OD pattern, which consisted of densely labeled regions alternating with relatively unlabeled regions, only 4 days after a monocular injection (1 mg in 20 ul saline). In addition, our procedure for flattening the cortex allows large extensions of the OD pattern in areas 17 and 18 to be visualized in a single section, thereby permitting us to reconstruct the entire pattern from only 3-5 sections.

We studied normal cats as well as cats reared with strabismus or monocular deprivation (MD). In both normal and strabismic cats, the label accumulates in the cortex in isolated patches or branching bands whose shapes are highly irregular. The boundaries of these labeled regions appear somewhat sharper in cats exposed to a prolonged period of strabismus. In MD cats, the labeled regions observed following injection of the deprived eye are more fragmented and much smaller in size, so that they appear dot-like over all of area 17. Although our results indicate that significant input from the deprived eye remains after almost 2 years of MD, preliminary analysis suggests that the total cortical area occupied by this input is noticeably less than that occupied by either eye of normal or strabismic cats. In all cats, the labeled pattern tended to be more filled-in in the contralateral hemisphere than in the ipsilateral hemisphere and, in both hemispheres, there was a tendency for regions of area 17 containing the peripheral visual field representation to be more filled-in than those representing the central visual field. In contrast to the monkey, the overall pattern of OD columns in area 17 of the cat is irregular and cannot be described as easily. In area 18, labeled regions are wider and more bar-like than those in area 17, and their orientation is roughly perpendicular to the 17/18 and 18/19 borders. While area 19 appears relatively unlabeled, regions of moderate labeling are observed in visual areas located in the medial bank of the lateral suprasylvian sulcus.

Supported by EY02193, EY05621, BNS-8200083.

267.5 THE EFFECT OF DARK-REARING UPON cAMP-METABOLISM IN THE VISUAL CORTEX OF CATS. C. Aoki* & P. Siekevitz. Lab. of Cellular Biology, The Rockefeller University, New York, N.Y. 10021.

The evidence from many labs indicate that the neuronal plasticity of the postnatally developing visual cortex is dependent on visual experience. Recently, Kasamatsu et.al. have reported evidence indicating that this form of plasticity is influenced by the local concentration of l-norepinephrine (NE) in the visual cortex. Further, Cynader's group has reported that the plastic period, which spans the first 3 to 4 months after birth, can be extended at least a year, so long as the animal is completely deprived of visual experience during that time. Our goal was to study the molecular mechanism by which NE modulates visual cortical plasticity of this type. We have examined in homogenates from areas 17 and 17 plus 18 of the visual cortex the ontogenetic changes of three enzymes involved in cAMP metabolism, namely 1)cAMP- and cGMP-phosphodiesterase (PDE); 2)cAMP-dependent protein kinase and 3)adenylate cyclase. In addition, we have studied the protein species phosphorylated by the kinase. The kittens used were reared under three different conditions: 1)light-reared(LR); 2)dark-reared (DR) and 3)dark-reared followed by light-reared condition (DLR), and sacrificed at the same time of day.

During the 6 mos. postnatal period, both cAMP-PDE and cGMP-PDE specific activities increased 2½-3 fold. No difference was observed on the cGMP-PDE among the LR, DR and DLR animals. A significant decrease in cAMP-PDE was observed at 2 mos. in the LR but not in the DR or DLR kittens. Protein kinase activity was determined by examining autoradiograms of the phosphorylated proteins after ^{32}-P-ATP incubation. Differences were observed in the basic phosphorylation pattern during development and in the pattern between LR and DR animals. A cAMP-dependent protein kinase was present in the LR, DR and DLR visual cortex. The addition of cAMP caused an increase in the phosphorylations of synapsin I under all the rearing conditions. The addition of calmodulin caused an increase in the phosphorylations of 51,000M_r and a 60,000M_r protein under all the rearing conditions. The adenylate cyclase of the visual cortex was similar to the activity in other tissues in being activated by GTP, Mn^{2+} and GppNHp and by dopamine and NE. The specific activity in LR kittens was very low at birth and increased to adult levels by 4-mos.; the percentage activation by the activators was the same in 5-day to 6-mos. tissues. The activity in DR kittens is being studied. The possible role of cAMP in visual cortical plasticity will be discussed.

Supported by a National Institutes of Health National Research Service Award No. 7524 from the National Institutes of General Medicine Sciences.

267.6 CLONIDINE AND VISUAL CORTICAL PLASTICITY: NEW EVIDENCE FOR NORADRENERGIC INVOLVEMENT. S.B. Nelson*, Marjory Schwartz*, and J.D. Daniels, Division of Engineering and Center for Neural Sciences, Brown University, Providence, RI 02912.

We have shown previously that acute but not chronic administration of 6-hydroxydopamine is capable of preventing the visual cortex ocular dominance plasticity normally expected after monocular deprivation in kittens (Bear et al. Nature 302:245 (1983)). Those results, and the fact that non-plastic adult cats have significant levels of cortical norepinephrine (NE) indicate that plasticity cannot be correlated solely with tissue NE levels. We believe NE's facilitatory effect on plasticity may require elevated transmitter release and post-synaptic receptor response. To investigate the importance of transmitter release we studied monocularly deprived kittens treated with the alpha-2 adrenergic agonist clonidine (CLON). CLON inhibits firing of noradrenergic locus coeruleus neurons and hence inhibits release of NE in cortex.

CLON (400 ug/kg) was administered by i.p. injections given every 4 hours beginning 2 days prior to lid suture and continuing through a 5 day period of monocular deprivation. In one litter, three kittens all received CLON, in 2 other litters, 3 kittens received CLON and 2 kittens given an identical schedule of saline injections served as controls. For these last five kittens, recording was performed blind and the drug code was broken only after final histograms were generated.

In the control animals, 73% of the units (N=104) were driven predominately by the open eye, while only 16% were driven by the deprived eye. In the CLON treated animals, the number of units driven by the open and deprived eyes were nearly indentical, being 42% (N=218) and 39% respectively. Thus CLON treatment did prevent the expected ocular dominance shift seen in controls. We also found that CLON itself, in doses up to 800 ug/kg given i.v. during physiological recording, had no acute effect on single unit responses.

In order to insure that CLON was in fact inhibiting NE release, we assayed CSF levels of MHPG, the primary central NE metabolite. 0.3cc samples of cisternal CSF were withdrawn before, during and immdiately following CLON treatments. HPLC analysis revealed that in the 6 CLON treated animals, MHPG levels declined 48% over the 7 day period, from an average of 8.65 ng/ml to an average of 4.43 ng/ml.

Our results provide new evidence that NE can modulate plasticity in kitten visual cortex, and that proper release of the NE may be crucial for its action.

Supported by ONR Contract N00014-81-K-0136.

267.7 RESTORATION OF NEURONAL PLASTICITY IN CAT VISUAL CORTEX BY ELECTRICAL STIMULATION OF THE LOCUS COERULEUS. T. Kasamatsu, K. Watabe, E. Schöller* and P. Heggelund.* Div. of Biol., Calif. Inst. Tech., Pasadena, CA 91125, USA; and Neurobiol. Lab., Univ. of Trondheim, Trondheim, N-7055, Norway.

We have studied a role of central norepinephrine (NE)-containing system in regulating neuronal plasticity in cat visual cortex. We use changes in the distribution of ocular dominance as a simple yet reliable assay of plasticity present in a given cortex. Previously, we manipulated function of NE terminals within the visual cortex by direct perfusion of either a catecholamine (CA)-related neurotoxin, 6-hydroxydopamine (6-OHDA) or exogenous NE or both. Chemical degeneration of CA terminals due to 6-OHDA led to a loss of neuronal plasticity. The cortical sensitivity to alterations in visual experience was restored by the NE perfusion into either the kitten cortex pretreated with 6-OHDA or the adult visual cortex which had lost its plasticity due to outgrowing of the postnatal susceptible period.

In the present study, we wanted to show the restoration of visual cortical plasticity in old kittens (17~ wks old) and adult cats by direct stimulation of NE cells in the locus coeruleus (LC), thus leaving the cerebral cortex totally intact. The animals were monocularly exposed to a laboratory environment in the light for 2 hrs everyday during which time the LC was electrically stimulated by a train of 4 pulses (0.05 msec width, 50 Hz, every 3.3 sec, ~1.5 mA). Otherwise, the animal was housed in the dark. This brief monocular exposure combined with LC stimulation lasted for 6 days, attaining a total of 12 hrs of monocular experience.

When we recorded from these animals on the 7th day, we found the significantly lower (30%~) number of binocular cells than normal. The plasticity-restoring effects of LC stimulation were mediated through NE terminals in the visual cortex, since no such effects were obtained if the visual cortex had been priorly perfused with 6-OHDA. Some animals were revived from the first recording, returned to the preceeding routine without accompanying electrical shocks to the LC and recorded a second time after variable time intervals. There was a sign of gradual disappearance of the restored plasticity at a rate of a 21% loss every 7 days. However, in animals subjected to the usual form of monocular lid suture and kept in our cat colony (17L/7D cycle) after reviving from the first recording, the altered distribution of ocular dominance sustained or binocularity further decreased. The present paradigm in restoring plasticity was free from nonspecific actions of exogenous chemicals as well as some pathology due to the chronic implantation of a metallic cannula directly into the cortex. These results strengthened the NE hypothesis of visual cortical plasticity. Supported by EY-03409

267.8 THE SENSITIVE PERIOD FOR MODIFYING VISUAL CALLOSAL CONNECTIONS IN THE RAT. R. Malach*, J. Olavarria* and R.C. Van Sluyters (SPON: J.F. Metcalf). School of Optometry and Neurobiology Group, University of California, Berkeley, Ca 94720.

In the normal mature rat, visual callosal connections are distributed tangentially in a band that runs along the 17/18 border and in several rings that lie in extra-striate cortex. Enucleation of one or both eyes at birth results in the development of an altered pattern of callosal connections. The most striking effect of monocular enucleation (ME) is the appearance of an additional callosal band, situated roughly in the center of area 17, in the hemisphere contralateral to the enucleated eye, while binocular enucleation (BE) leads to the appearance of acallosal regions within the 17/18 band and irregularly shaped extensions that invade well into area 17. We have shown that, in the rat, the pattern of callosal connections achieves its adult form by 12 days of age, and that enucleation at birth alters the pattern of normal development without changing its time course (Olavarria, et al, Invest Ophthal, 24, ARVO Abst Suppl, 9, 1983).

In the present study we investigated the effect of delaying the time at which enculeation was performed in order to characterize the sensitive period for modifying the pattern of callosal connections. Fifty pigmented rat pups were subjected to ME or BE at ages ranging from birth to 11 days. After a survival period of several weeks, one cortical hemisphere received multiple injections of horseradish peroxidase (HRP) spread evenly across the cortical surface. The rats were perfused after 24 hrs, and the opposite hemisphere was flattened and sectioned tangentially. Sections were reacted for HRP histochemistry to reveal both anterogradely and retrogradely transported label. Our results indicate that the sensitive period for modification of callosal development ends by 6 days of age in both BE and ME rats, since rats enculeated at this age develop a pattern of callosal connections that is indistinguishable from normal. It is important to note that this sensitive period ends at a time when development of callosal connections is far from complete. Indeed, in 6-day-old normally reared littermates of these enucleated pups, the distribution of callosally projecting cells was clearly immature -- large numbers of filled cells still were present throughout area 17. Pups enucleated at 5 days of age develop a pattern of connections that is partially disrupted, while pups enucleated at 4 days of age exhibit a degree of abnormality comparable to that found in pups enucleated at birth, suggesting that the sensitive period for modifying callosal connections in the rat terminates rather abruptly. Possible factors involved in determining the duration and characteristics of this sensitive period will be discussed.

Supported by EY02193 and BNS8200083.

267.9 Corpus Callosum and Development of Striate Binocularity: Complete Critical Period Within Three Postnatal Weeks. Andrea J. Elberger. Dept. of Neurobiology and Anatomy, Univ. of Texas Medical School at Houston, Houston, Texas 77025, and Earl L. Smith, III* College of Optometry, Univ. of Houston, Houston, Texas 77004.

Abnormal visual experience during development has been shown to result in alterations of the visual system. For example, induced conditions such as strabismus, monocular deprivation, astigmatism, and anisometropia result in reduced levels of striate binocularity, as well as deficits in visual acuity (amblyopia). The period of time within which the developing visual system is susceptible to abnormal visual experience is known as the critical period. For these particular conditions the critical period extends from the second week through 4-6 months, postnatally. Previously, section of the corpus callosum (CC) was also shown to affect the normal development of the visual system, however the critical period for callosum section to produce amblyopia was limited to the first postnatal month (Elberger, Soc. Neurosci. Abstr. 82.2, 1982). To more fully investigate the possibility that the callosum may have a different critical period than that for abnormal visual experience, we explored the length of time that callosal section has an effect on the development of cortical binocularity.

To date, we have examined 30 cats which had the posterior CC sectioned between 13 days and 14 weeks after birth. We have performed extracellular single unit recording in striate cortex within 2 years after the surgery, with a minimum time between surgery and recording of 10 weeks in the 14 week CC cats. Within each age group the results were consistent and demonstrated an extremely restricted period of time during which CC section had any effect on altering the level of striate binocularity. The greatest change in ocular dominance was in the 13 day CC cats, with a minimum of 20% of the cells retaining binocular activation. The proportion of binocularly excited neurons gradually increased in the 14, 15 and 18 day CC cats. By 19 days after birth, CC section no longer resulted in any abnormal distribution of ocular dominance. For 17 cats with CC section at 19 days-14 weeks the proportion of monocularly activated cells averaged 10-17%. In all groups of cats, all other receptive field parameters were qualitatively normal.

The results show a distinct critical period for corpus callosum to influence the development of cortical binocularity, which is remarkably similar to that for the development of acuity. This callosal critical period is the first three postnatal weeks. During this time period, CC section produces the same range of alterations of binocularity and acuity as does abnormal visual experience. However, the critical period for the latter is much longer, and hardly overlaps the critical period for the corpus callosum.
Supported by Grants MH36526 to A.J.E. and EY03611 to E.L.S.

267.10 THE SENSITIVE PERIOD FOR THE LOSS OF BINOCULAR DEPTH PERCEPTION IN KITTENS. Brian Timney. Department of Psychology, University of Western Ontario, London, Canada, N6A 5C2.

Kittens' binocular depth discrimination abilities improve rapidly during the sixth and seventh week, at a time when cortical disparity-tuned neurones are acquiring adult-like characteristics (Timney, 1981). Recently, I showed that periods of monocular deprivation lasting from the time of natural eye opening until 35 days or older resulted in a complete breakdown of binocular depth perception. Prolonged deprivation imposed after the age of four months had no effect (Timney, 1983), suggesting the existence of a sensitive period occurring during the first few months of life. The present study was designed to obtain a profile of that sensitive period.

Kittens were subject to 10-day periods of monocular deprivation beginning at different ages. Deprivation was accomplished by suturing the eyelids of one eye using conventional procedures. The deprivation periods were begun at the ages of 30, 40, 50, 60, 70 and 80 days. After a recovery period following eye opening monocular and binocular depth thresholds were obtained for each kitten using the jumping stand technique. Briefly, the kittens were trained to jump from a platform toward the closer of two patterned surfaces. Normal kittens can do this under binocular viewing conditions with virtually no training and typically can discriminate separations corresponding to less than 5 minutes of retinal disparity. When viewing monocularly, performance is almost invariably much worse, allowing the inference that normal kittens possess stereopsis.

In the present experiment, deprivation from 30 to 40 days led to generally poor binocular performance, although binocular thresholds were slightly better than monocular. Deprivation between 40 and 50 days completely abolished binocular superiority, but the effect was progressively less pronounced as deprivation was applied at later ages. By 70 days the deprivation had little effect. These results indicate that the sensitive period begins between days 30 and 40 and is almost completely over by day 70. Unlike resolution acuity, the impairment of stereoacuity appears to be permanent. Studies are now underway to determine the minimum amount of deprivation necessary to cause a breakdown in binocular depth perception.
Timney, B. Invest. Ophthalmol. Vis. Sci. 1981, 21, 493-496.
Timney, B. Developmental Brain Research 1983, 7, 235-243.
Supported by grant #MA7125 from the Medical Research Council of Canada.

267.11 BINOCULAR INTERACTION IN CORTICAL CELLS OF MONOCULARLY DEPRIVED KITTENS. R. D. Freeman and I. Ohzawa*. School of Optometry, University of California, Berkeley, CA 94720.

Several lines of evidence suggest that some input from the deprived eye to visual cortex remains following monocular deprivation. In previous work, we attempted to find evidence of such input using sinusoidal gratings presented to each eye so that one was phase shifted with respect to the other (Freeman & Robson, Exp. Brain Res. 48:296-300, 1982). For normal cats, this stimulation procedure generally results in marked phase specific binocular interactions involving both facilitation and inhibition. But we did not observe these effects in cats that were studied following long-term monocular deprivation.

In the current investigation, we have pursued the possibility of binocular interaction in monocularly deprived kittens by using relatively brief periods of deprivation (from 3 to 21 days). For single-unit study, we plotted receptive fields for each eye if possible and estimated ocular dominance qualitatively. Next, we conducted quantitative tests of orientation and spatial frequency tuning using sinusoidal gratings. Finally, we determined responses to binocular stimulation using optimal grating parameters. Relative spatial phase was altered to one eye and the set of phases was interleaved randomly with monocular presentations to each eye so that direct comparisons could be made between monocular and binocular responses.

Our results show a variety of binocular interactions. First, a large proportion of cells that appeared monocular or nearly so were influenced by input from the other eye. This influence was often phase specific especially for simple cells. In other cases, phase non-specific suppression was observed. Suppression of the non-deprived eye by the deprived eye was observed more frequently than the reverse case. However, this bias may simply reflect the effects of the rearing procedure which produces an ocular dominance shift to the non-deprived eye. Interestingly, phase non-specific suppression was also observed when the stimulus to the silent eye was orthogonal to the preferred orientation of the dominant eye indicating that the effect was not orientation specific. For binocular cells, a full range of phase-sensitive responses were observed and these were not obviously abnormal or distinguishable from responses found in the normal kitten or adult cat. We conclude that during the early stages of monocular deprivation, binocular connections remain which cannot be demonstrated using monocular excitation.
(EY01175)

267.12 DARK REARING DOES NOT PROLONG SUSCEPTIBILITY TO THE ANATOMICAL EFFECTS OF MONOCULAR DEPRIVATION. G.D. Mower and C.J. Caplan*. Dept. of Neurology, Children's Hospital, Boston, Ma. 02115.

Several recent studies have shown that dark rearing extends the period of susceptibility to the physiological effects of monocular deprivation (MD) in visual cortex. Does dark rearing also prolong susceptibility to the anatomical effects of MD?

Eye injections of tritiated proline were used to label ocular dominance (OD) columns in cortical layer IV. Studies were done in cats who experienced only prolonged (4 mos) dark rearing (DR cats, N=2) and in cats who experienced 3 mos of MD after 4 mos of dark rearing (DR-MD cats, N=4). Results from these cats were compared with those from normal (N=2) and MD (N=2) cats.

In DR cats, there was little evidence for OD columns. Label was nearly continuous in layer IV with only very slight fluctuations in grain density. In normal cats, OD columns were clearly evident as alternating patches of high and low grain density. This result suggests that visual input is necessary for normal segregation of geniculocortical afferents.

The DR-MD cats allowed us to address two issues: 1) can the overlapping afferents (after dark rearing) segregate into OD columns with delayed visual experience? 2) will such delayed segregation reflect the nature of the visual input? Surprisingly, injection of either the open (N=2) or the closed (N=2) eye in DR-MD cats resulted in uniform label throughout layer IV, similar to the pattern seen in DR cats. MD cats showed the expected expansion of columns from the open eye and contraction of columns from the closed eye. Measurements of cell sizes in the lateral geniculate nucleus indicated marked shrinkage of cells innervated by the closed eye in MD cats, but no shrinkage in DR-MD cats. Single unit recordings in area 17 of both DR-MD and MD cats showed a dramatic takeover by the open eye. These results suggest that delayed physiological plasticity in DR-MD cats is not paralleled by delayed anatomical plasticity.

Overall, it appears that formation of OD columns requires visual input occurring during early life. Dark rearing prolongs susceptibility to the physiological but not the anatomical effects of MD.

268.1 SIMULTANEOUS MONITORING OF ACTIVITY OF MANY NEURONS IN BUCCAL GANGLIA OF PLEUROBRANCHAEA AND APLYSIA. L.B. Cohen and H.S. Orbach. Dept. of Physiology, Yale University School of Medicine, New Haven, CT 06510.

Optical methods, using potential sensitive dyes, might allow simultaneous monitoring of the activity of all the neurons in ganglia with a few hundred neurons. Pleurobranchaea californica and Aplysia californica have interesting behavior and have the relatively large neurons that are necessary for optical measurements. In previous experiments, poor dye penetration of the connective tissue sheath caused difficulty. Dyes that penetrated and stained routinely in nudibranchs did not stain every Pleurobranchaea preparation and did not stain Aplysia at all. We find that staining is better with the dimethin analogue, 433, of the merocyanine dye, XVII (Ross et al., 1977), and that reducing the divalent ion concentration by 50% during staining also improved the results.

In preliminary experiments carried out on dissected buccal ganglia of Pleurobranchaea, stimulation of the stomatogastric nerve resulted in rhythmic bursts of action potentials recorded from roots 1 and 3 that are correlated with rhythmic movements of the buccal mass (Davis et al., 1973). Simultaneous optical measurements detected activity in 20-30 neurons. Some neurons had activity that was phase locked to the burst on the roots, other neurons had activity that was correlated with the stimulus, while activity in a third group of neurons did not seem to be correlated with either the root bursts or the stimulus. Preliminary experiments on Aplysia ganglia showed that optical signals could be detected from many neurons in response to electrical stimulation of buccal nerves.

We hope that the technique can be developed to the point that all the neurons active during feeding can be monitored simultaneously. Supported by NIH Grant NS08437.

268.2 THE HISTAMINERGIC NEURON C2 OF APLYSIA IS A MECHANOAFFERENT CELL WHOSE OUTPUT CAN BE GATED SYNAPTICALLY. K.R. Weiss, H.J. Chiel and I. Kupfermann. Center Neurobiol. & Behav., NY State Psych. Instit.; Depts of Physiol., Anat.& Cell Biol., and Psychiat., Columbia P&S, and Schl. Dent. & Oral Surgery, N.Y.

The histaminergic cell C2 provides excitatory input to the MCC, a neuron that mediates aspects of food arousal. In a semi-intact preparation C2 fires when food is applied to the lips and contributes to the activation of the MCC. Activity of C2 occurs each time that the jaws close, and is not dependent on the continued presence of food as long as rhythmic buccal movements persist. The spiking of C2 is always preceded by prepotentials that are axon spikes conducted from the periphery and are blocked at a point electrically distant from the soma. The A spikes were reduced in size by hyperpolarizing the soma, and simultaneous intracellular and extracellular recording revealed that mechanical pressure stimulation of the mouth elicited spikes, each of which appeared first in the nerve, followed by an A spike in the soma. Mechanical stimuli were effective in high Mg^{++} low Ca^{++}, indicating that C2 may be a primary mechanoafferent neuron. Since the A spikes show temporal and spatial summation, they are functionally similar to EPSPs. Functional meaning of this mechanism is suggested by the finding that C2 receives powerful inhibitory input that can prevent the A spikes from bringing the cell to the threshold for soma spikes. Furthermore, recording from C2 and the MCC or other followers revealed that synaptic output of C2 only occurs when a full soma spike is triggered. We found that at the point of trifurcation of the axons of C2, the main axon rapidly increases in diameter, creating a region of low safety. Synaptic output of C2 occurs between the trifurcation and the cell body. Thus, spikes which fail at the trifurcation can not produce synaptic release. In summary, C2 is a highly unusual proprioceptive cell that is normally in a state in which it fails to pass information into the nervous system. If its axon input occurs at a high rate, it switches into a transmitting mode and passes the proprioceptive information to an arousal system, as well as to cells involved in motor patterning (Chiel et al.). Synaptic input operating at a central locus can function to modulate the level at which this gate opens. [Grants 1 ROl MH36730, 5 ROl MH35564]

268.3 AN IDENTIFIED HISTAMINERGIC NEURON ACTS PRE- AND POST-SYNAPTICALLY TO INHIBIT THE OUTPUTS OF IDENTIFIED BUCCAL-CEREBRAL INTERNEURONS. Chiel, H.J. Weiss, K.R. and Kupfermann, I. Center Neurobiol., Behav., NYPI; Depts of Physiol., Anat.& Cell Biol., and Psychiat., Columbia P&S, and Schl. Dent. & Oral Surg., New York, NY 10032.

Feeding in Aplysia requires the coordinated activity of the buccal and cerebral ganglia. We have identified two buccal interneurons, B17 and B18, (buccal-cerebral interneurons, BCIs) that may participate in this coordination, since they send axons into the buccal-cerebral connective, and provide monosynaptic excitatory or inhibitory inputs to the cerebral ganglion. We found that the identified histaminergic neuron, C2, which is a cerebral mechanoafferent cell (Weiss et al., these abstracts), modulates the outputs of the BCIs. C2 and the buccal cells are reciprocally connected. The BCIs produce monosynaptic IPSPs in C2, and, in turn, C2 reduces the size of the PSPs that the BCIs evoke in cerebral neurons. C2 acts in two ways: 1) it polysynaptically inhibits the spiking of BCIs, which results in all or none block of the PSPs, and 2) it monosynaptically inhibits the PSPs in a graded fashion. To study the mechanism of the graded inhibition, we determined the coefficient of variation of control PSPs and of PSPs that were reduced in size by firing C2. The reduced PSPs showed an increased coefficient of variation. Estimates of m and q based on this analysis suggest that firing of C2 can produce a reduction of quantal number (m) of 50-80%, while quantal size (q) remains virtually unchanged. Thus, the reduction in the PSP is not due to a postsynaptic factor that produces a constant percentage reduction of PSP size, e.g., an increase of postsynaptic membrane conductance, and is therefore likely to be due to presynaptic inhibition. Since the BCI as well as C2 are active during the generation of "feeding motor programs" induced by tonic stimulation of an esophageal nerve, and C2 is activated during feeding-like behavior in a semi-intact preparation (Weiss et al., these abstracts), these cells may contribute to coordination of functional activity between the buccal and cerebral ganglia during feeding. [Supported by 5 T32 MH15174, 1 ROl MH36730, 5 ROl MH35564.]

268.4 SEROTONIN CONTROLS FEEDING BEHAVIOR IN THE MEDICINAL LEECH. C.M. Lent, M.H. Dickinson* and C.G. Marshall* Center for Neuroscience, Brown University, Providence, R.I. 02912.

We investigated the effects of serotonin (5-HT) upon feeding behavior by Hirudo medicinalis. Three behaviors are significantly altered after a bath in 5-HT (3×10^{-5}M, 20 min): 1) leeches initiate swimming toward vibration with a shorter latency, 2) they bite a 35°C surface more frequently, and 3) they ingest larger blood meals than controls. Exposing dissected leeches to micromolar 5-HT produced four physiological responses: jaw movements resembling biting, a constriction of the crop, an increase in secretion of saliva from between the paired teeth on the jaws, and a rhythmic pharyngeal peristalsis. Salivation and peristalsis are peripheral effects, and both the volume of saliva, as well as the frequency and strength of the peristalsis, increase with 5-HT concentration (10^{-8} to 10^{-6}M). Thus, 5-HT enhances feeding behavior and activates some of its physiological systems.

We examined the electrophysiological responses of identified serotonin-containing neurons in the C.N.S. to inputs and outputs which could be associated with feeding. Segmental sensillae bear hair cells which detect water vibration (Young, S., et al., J. Comp. Physiol. 114:111, 1981), and their mechanical stimulation synaptically excited the Retzius cells (RZ) in segmental ganglia. Thermal stimulation of the prostomium synaptically excited RZ within the subesophageal ganglion (SubEG) substantially increasing their impulse frequency. The intracellular stimulation of RZ within the SubEG usually produced salivation. Further, stimulation of the large, lateral (LL) cells within the anterior SubEG, which contain 5-HT, reliably produced pharyngeal contractions. Thus, serotonin neurons are excited to higher impulse levels by stimuli which evoke feeding, and increasing their impulse activity produces salivation and pharyngeal pumping.

We attempted to reduce 5-HT levels in the leech by injecting them with 5,7 dihydroxytryptamine. Six of 9 injected leeches would neither bite a warm surface nor feed. Glyoxylic acid histochemistry revealed that all the peripherally projecting, 5-HT cells (RZ & LL) were selectively and completely ablated in the non-feeding leeches. Bathing these depleted leeches in 10^{-4}M 5-HT for 10 min, restored their biting behavior. Well-fed leeches do not bite or feed and 5-HT exposure restores their biting behavior as well. Thus, biting behavior can be restored in leeches whose behavior has been abolished by pharmacological or physiological methods by exposure to exogenous serotonin.

Serotonin has a central role in organizing and controlling the complex feeding behavior of the medicinal leech. (Supported by NIH grant NS-14482 and NSF grant BNS 79-15108 to C.M.L.).

268.5 THE SLUG LIMAX MAXIMUS SHOWS POST-INGESTIVE FOOD AVERSION LEARNING TO AMINO ACID DEFICIENT DIETS. K. Delaney* & A. Gelperin, Dept. Biology, Princeton University, Princeton, NJ 08544 and Bell Laboratories, Murray Hill, NJ 07974.

The capacity of a generalist herbivore, the pulmonate mollusc Limax maximus, to modify its feeding behaviour on the basis of post-ingestive cues was examined. The storage of a sensory representation of a diet for several hours which can occur with this type of learning may provide an opportunity for examination of cellular mechanisms of short term memory.

An artificial diet which contained free amino acids as the nitrogen source was suspended in agar and presented to cultured slugs kept individually in 15 cm Petri dishes. The ratios of the free amino acids present approximated those reported for cabbage, a food normally eaten by Limax. Deficient diets differed from the complete diet in that they lacked a single "essential" amino acid, e.g., methionine. An aversion was assessed on the basis of how much diet was eaten by animals given the deficient diet alone versus animals given only the complete diet. In addition, the amount of diet consumed by "naive" slugs was compared to that consumed by slugs after they had experienced the diet for at least 7 days. The latency to feed was assessed by examining the number of slugs which had eaten a portion of the diet within 2-2.5 hrs of its presentation. Initially, the amounts of deficient & complete diets eaten were equivalent. While control animals maintained or increased the amount of complete diet they ate over the course of the 7 days, animals provided with the deficient diet reduced their consumption and increased their latency to feed. Using groups of only 6-8 animals, significant differences in consumption between slugs eating complete and deficient diets were found by the second day of feeding. By the third day, if a meal was eaten by animals fed the deficient diet, it was only 30-40% of the amount eaten by control animals. The amount of a third diet, rat chow in agar, eaten by animals previously provided with the amino acid deficient diet for one week was greater than or equal to that eaten by control animals. This indicates that the depressed appetitive and consummatory behaviour of slugs fed the deficient diet was specific to the deficient diet. Animals which had developed an aversion to the deficient diet would also initially show an aversion to the complete diet but, after an initial small meal, they increased their consumption on subsequent feedings to a level equal to that of animals fed only the complete diet. Animals fed the complete diet and then switched to the deficient diet consumed the deficient diet initially as if it were complete but then reduced their consumption over several days. Animals returned to a diet of rat chow after one week on the deficient diet retained a significant aversion to the deficient diet for more than 2 weeks.

268.6 CHANGES IN SPECIFIC INTERNEURONS PRESYNAPTIC TO COMMAND NEURONS UNDERLYING ASSOCIATIVE LEARNING IN PLEUROBRANCHAEA. J. A. London and R. Gillette, Dept. of Physiology and Biophysics, Univ. of Ill., Urbana, IL 61801

Food avoidance conditioning of the predatory marine slug, Pleurobranchaea californica, using a paired food-shock paradigm causes suppression of the paracerebral feeding command neuron (PCNs) activity in response to food (Davis and Gillette, 1978). These command neurons are usually excited by food and act to initiate and sustain feeding (Gillette, Kovac and Davis, 1978); in conditioned animals, food stimuli cause tonic inhibition of the PCNs via a prolonged barrage of IPSPs. We previously documented a pathway mediating cyclic inhibition to the PCNs during feeding activity. This pathway is composed of serially connected interneurons driven both by chemosensory inputs and cyclic activity in the feeding oscillators (London and Gillette, 1983). The Interneuron 1s (Int-1s) make monosynaptic inhibitory connections on the PCNs. We find that Int-1s are active during food stimulation of conditioned animals and that hyperpolarization of a single Int-1 reduces the inhibition seen in the PCNs, indicating their role in the learned suppression of feeding behavior. A second population, the Int-2s, potently excite the Int-1s and synchronize Int-1 activity. Hyperpolarization of a single Int-2 in trained animals decreases the amplitude and duration of PCN inhibition in response to food. The excitatory depolarization of the neurons following measured food stimulus application persists significantly longer in conditioned that in control subjects. An analysis of variance comparing the amplitude, duration, and area under the depolarization of conditioned versus naive animals showed significant difference between the groups ($p < 0.001$). A Scheffe test of the means indicated that the basis of the difference was due to the duration and the area of the depolarization response. The averaged response duration for naive animals stimulated by 0.5 ml of squid homogenate applied to the oral veil over 10 seconds was 10.9 ± 1.3 s; that for conditioned animals was 19.4 ± 1.7 s ($p < 0.001$). The response area was 281 ± 17.8 mV·Sec for naive and 516.8 ± 21.3 mV·Sec for conditioned animals ($p < 0.001$). A comparison of the amplitudes showed no significant difference, suggesting that there is no change in the early sensory input. The mechanisms underlying the learning-induced alteration in Int-2 responsiveness are under investigation.

This research was supported by NSF BNS 79-18329 to R. G. and NIH GM07283 to J.A.L.

268.7 MOTIVATION TO FEED AFFECTS ACQUISITION OF FOOD-AVOIDANCE CONDITIONING IN PLEUROBRANCHAEA. Martha L. U. Gillette, J. A. London and Rhanor Gillette. Dept. of Physiology and Biophysics and Neural and Behav. Biol. Prog., University of Illinois, Urbana, IL 61801.

Motivation has a particularly significant effect on task performance in learning situations. A distinct inverted-U shaped relationship between arousal (the motivational variable) and task performance characterizes mammalian learning (Yerkes and Dodson, 1908; Hokanson, 1969). Mammals which are at extremes of the arousal state show low motivation to learn while those partially aroused acquire the task relatively rapidly. The neurophysiological mechanisms underlying these motivational variables have remained unamenable to analysis in these complex organisms. The simple nervous system of the marine mollusc Pleurobranchaea has been useful for examining reflections of motivation in feeding command cells as well as the central neuronal changes underlying food-avoidance conditioning (Davis and Gillette, Science, 1978; Gillette and Gillette, J. Neurosci., 1983; London and Gillette, this volume). We report here that Pleurobranchaea shows motivational affects on task acquisition similar to mammals.

We were alerted to this phenomenon while performing food-avoidance conditioning studies in which animals were observed for acquisition and retention of the task over a period of weeks. Some animals initially would not feed. When retested after one week, they fed with moderate feeding thresholds (as determined by that concentration of food which elicited feeding); they also acquired the task more rapidly than animals with low thresholds. Upon dissection, these animals were found to have food in the posterior gut suggesting that initially they had been satiated. A more careful examination of the phenomenon demonstrated an inverted U-shaped relationship between both feeding experience and threshold vs task acquisition: animals which were either fed to satiation (very high threshold) or held without feeding (very low threshold) were much poorer at acquiring the food-avoidance task than those which had been partially fed and showed moderate thresholds for food. In fact some animals acquired aversion to food in only one food-shock trial. These results suggest an adaptive relation for motivation in learning. For animals which are satiated and **not** aroused by food or those voraciously hungry and overaroused by food, it may be maladaptive to associate food with a negative simulus. However, in animals aroused by food stimuli but not starving, the adaptive value inherent in feeding may be less important than the negative stimulus with which it is associated. The neurophysiological correlates of these phenomena are under investigation. Supported by NSF BNS 79-18329.

268.8 HABITUATION CORRELATED WITH A SHIFT IN THE VOLTAGE DEPENDENCE OF MECHANORECEPTOR CHANNELS IN THE PROTOZOAN, STENTOR. D.C. Wood, Psychobiology Program, Department of Psychology, University of Pittsburgh, Pittsburgh, PA 15260.

The ciliate, Stentor coeruleus, contracts to 40% of its extended length in 10 msec after application of a suprathreshold mechanical or electrical stimulus. During repeated mechanical stimulation the probability of contracting progressively decreases. This decrement in response probability exhibits 7 of the 9 parametric characteristics of habituation but does not show dishabituation.

Mechanical stimuli elicit graded (<25 mV) receptor potentials which are capable of triggering action potentials. Action potentials trigger contractions. When recording at room temperature repeated mechanical stimulation produces a progressive decrement in receptor potential amplitude which is highly correlated with the decrement in probability of an action potential occurring. Action potential amplitude is not altered.

Records from cells cooled to 10°C typically reveal receptor potentials (<15 mV) which are insufficient to elicit action potentials. Under these circumstances repeated mechanical stimulation does not produce a decrement in the receptor potential. Similarly repeated elicitation of action potentials using depolarizing current pulses does not produce a receptor potential decrement. However, if the mechanical stimulus is following in 200 msec by a current elicited action potential then the receptor potential undergoes a progressive decrement. Thus habituation of the mechanoreceptor potential appears to be contingent on action potential production.

Repeated presentation of combined mechanical and electrical stimuli also produces a significant reduction in mechanoreceptor current. This current reduction was characterized by generating I-V plots for cells before and after habituation. Before habituation mechanoreceptor currents have a reversal potential at +20 mV and their conductance is voltage dependent reaching a maximum at -10 to +20 mV while being only 25-40% maximal at resting potential (-50 to -55 mV). After habituation the reversal potential and maximal conductance are not significantly altered but the curve for the voltage dependent conductance is shifted to depolarized values. At resting potential the conductance is now less than 20% maximal. This shift indicates that the unresponsive form of the mechanoreceptor channel which is normally present at hyperpolarized potentials has become more prevalent. This conclusion is reinforced by the observation that significantly more labelled d-tubocurarine binds to habituated than to control cells, since d-tubocurarine has previously been shown to bind selectively to the unresponsive form of the mechanoreceptor channel.

268.9 IMMUNOCYTOCHEMICAL STUDIES OF NEURONS PRODUCING PRESYNAPTIC FACILITATION IN THE ABDOMINAL GANGLION OF APLYSIA CALIFORNICA H. B. Kistler, Jr., R. D. Hawkins, J. Koester, E.R. Kandel, and J.H. Schwartz. Center for Neurobiology & Behavior, Columbia College of Physicians & Surgeons and The New York State Psychiatric Institute, New York, N.Y. 10032.

Sensitization of the gill withdrawal reflex in Aplysia is a simple form of learning resulting in part from presynaptic facilitation of the LE mechanoreceptor neurons of the abdominal ganglion. Previous work has established that facilitation can be produced by application of serotonin, stimulation of the pleuroabdominal connectives or direct stimulation of members of the L29 cell cluster. In addition, earlier electron microscopic studies have shown that L29 cells are similar morphologically to known serotonergic neurons and take up ^{3}H-serotonin with high affinity. It therefore seemed likely that LE sensory neurons receive facilitatory serotonergic input from L29 cells.

Serial 16-um cryostat sections of 7 abdominal ganglia were examined using serotonin antiserum (provided by Harry Steinbusch, Free University, Leiden) and indirect rhodamine immunofluorescence. Serotonin-immunoreactive fibers bearing varicosities closely apposed both the cell bodies and the initial segments of physiologically identified sensory neurons marked by intracellular injection of Lucifer Yellow. Although these observations suggest that sensory neurons receive serotonergic innervation, the origin of these immunoreactive fibers is not the L29 cells. No immunoreactive cell bodies appeared on the ventral surface of the left hemiganglion, the location of L29 cell bodies, whereas identified serotonergic RB cells and several previously unidentified cell clusters were brightly immunofluorescent. Furthermore, when single physiologically identified L29 cells in 3 ganglia were marked with Lucifer Yellow, their cell bodies and processes exhibited no serotonin immunofluorescence. As a control, injection of dye did not interfere with serotonin immunofluorescence in cell bodies or processes of RB cells.

Although sensory cells appear to receive serotonergic innervation, these data indicate that it is not provided by the L29 cells. Our results are consistent with the idea that L29 neurons produce facilitation either by exciting as yet unidentified serotonergic interneurons or by acting in parallel with serotonergic facilitator neurons, using a related amine or another transmitter substance.

268.10 APLYSIA SIPHON SENSORY NEURONS SHOW TERMINAL-SPECIFIC FACILITATION. G. A. Clark* (SPON: E. R. Kandel). Center for Neurobiology and Behavior, Columbia Univ., and New York State Psychiatric Institute, New York, NY 10032.

Classical conditioning of the siphon withdrawal response in Aplysia involves activity-dependent enhancement of heterosynaptic facilitation at terminals of siphon mechanosensory cells onto siphon motorneurons. While activity-dependent enhancement can provide a mechanism for the stimulus and temporal specificity of classical conditioning, the mechanisms underlying response specificity remain unclear. In the present study, we examined the hypothesis that single siphon sensory cells can exhibit facilitation at synapses onto one set of followers without showing facilitation at synapses onto other followers. Although there is as yet no behavioral evidence that Aplysia exhibits response specificity, terminal-specific facilitation would provide a potential mechanism for conditioning one group of motor responses without affecting others.

To test for terminal-specific effects, we compared facilitation at two different sets of synapses of a single sensory neuron: one onto a central motorneuron (located in the abdominal ganglion), and the other onto a perpiheral siphon motorneuron (located on the distal end of the siphon nerve, several mm away). Central and peripheral neuropils were perfused independently, and monosynaptic excitatory postsynaptic potentials (EPSPs) evoked by intracellular stimulation of a siphon sensory cell (once every 40 seconds) were recorded in one central and one peripheral motorneuron. Following 10 trials, 0.1 mM serotonin (the putative transmitter of some of the facilitator interneurons) was perfused over either the central or peripheral neuropil, and washed out while testing continued. After 10 more trials, serotonin was perfused over the other neuropil.

Results showed that serotonin could facilitate transmission at either central or peripheral synapses independently. Application of serotonin onto the central neuropil caused a marked facilitation of EPSPs in central motorneurons, while peripheral EPSPs showed slight habituation from repeated stimulation of the sensory neuron (154% increase vs. 10% decrease, N = 10, $p < .05$). Similarly, application of serotonin onto the peripheral neuropil facilitated peripheral but not central EPSPs (62% increase vs. 10% decrease, N = 10, $p < .01$).

To determine whether activation of endogenous facilitators could produce terminal-specific facilitation, we stimulated the left abdominal connective (6 Hz, 4 sec) at the end of five experiments. Connective shock caused a striking enhancement of EPSPs in central but not peripheral motorneurons (251% increase vs. 6% decrease, $p < .05$).

Since both serotonin and connective stimulation can produce terminal-specific facilitation in siphon sensory neurons, we plan in future experiments to determine whether this mechanism is actually utilized by the nervous system to confer the types of response specificity evident during many forms of learning.

268.PO PERSISTENT CHANGES IN HERMISSENDA B PHOTORECEPTOR MEMBRANE PROPERTIES WITH ASSOCIATIVE TRAINING: A ROLE FOR PHARMACOLOGICAL MODULATION. A. McElearney* and J. Farley, Dept. of Psychology, Princeton U., Princeton, NJ 08544 (spon: S. Harnad)

Previous accounts of long-lasting conductance changes (e.g. I_A reduction; Alkon et. al., Science, 1982) produced in Type B photoreceptors by associative training with light and rotation have emphasized voltage-dependent inactivation and intracellular Ca^{++} accumulation as mechanisms responsible for persistent changes in Type B cell currents. Three lines of evidence suggest that pharmacological modulation of ionic currents - by a substance as yet unidentified within the optic ganglion - may also contribute to B cell membrane changes with training.

Simulation of light-rotation training sequences in the isolated nervous system by administering five simultaneous pairings of light and depolarizing current stimulation of the caudal hair cell produced significantly greater B cell depolarization and increased input resistances (R_m) than did pairing light with comparable direct current-produced depolarization of the B cell [8.2 ± 1.9 vs. 5.7 ± 1.1 mV; 45% vs. 29% MΩ increases; n=9; $p < .05$]. This indicates that the cumulative depolarization and enhanced input resistance which B cells normally undergo during pairings of light and rotation cannot be entirely reproduced by substituting current-induced depolarization for synaptically-induced depolarization of B cells. Perfusion of ligated B cells with a crude optic-ganglion homogenate resulted in a mean depolarization of 5 mV and an average increase in input resistance of 33% (n=8), while a crude extract made from the pedal ganglion had no consistent effect. The optic ganglion has previously been demonstrated to contain the type S/E cell which is the source of EPSPs in B photoreceptors.

As a preliminary step towards a thorough characterization of the pharmacological sensitivity of B cells and to delimit the possibilities of agents which may play an active role in mediating the effects of training in B cells, we have assessed the effects of various neuromodulatory agents upon membrane properties of Type B cells. In the dark, serotonin (30 μM; 5HT) produced an average 25% increase in input resistance and an average 4 mV depolarization of the Type B cells (9/9 cells; ranges 10-83%, -1 to +9mV), while octopamine and dopamine had no detectable effects in 6 and 11 different cells tested, respectively. 5HT also clearly enhanced both the peak and steady state light response of B cells. Norepinephrine produced a slow depolarization (mean = 3.1 mV, 6 cells) accompanied by decreased input resistances (mean = -10%), as did arginine-8-vasopressin (means = 2.8 mV, -14% % decrease in R_m; 5 cells).

268.PO TEMPORAL ORDER SENSITIVITY OF ASSOCIATIVE LEARNING IN HERMISSENDA. L. Grover* and J. Farley, Dept. of Psychology, Princeton U., Princeton, NJ 08544 (spon: B. Campbell)

Previous research has established close correspondences between many of the cardinal features of associative learning in vertebrates and the associative suppression of phototaxic behavior in Hermissenda produced by light-rotation pairings. These similarities include: pairing-, stimulus-, and contingency-specificity, reversibility, and both short- and long-term forms of memory. Our present results indicate a potentially important discrepancy, however. Unlike vertebrates, Hermissenda's most profound associative neural and behavioral changes occur when trained with light-rotation pairings involving simultaneous onsets and offsets. Forward pairings (i.e. light precedes rotation onset by 30 sec) produce no appreciable neural or behavioral change.

Exposure of intact animals to a standard 3-day associative conditioning procedure [50 light-rotation pairings/day; light intensity = 4.5×10^{-3} ergs. cm^{-2} sec^{-1}; rotation = 2.24g; each stimulus 30" in duration, ITI=2 min] in which light onset either coincided with (simultaneous), preceded by 30 sec (forward), or followed by 30 sec (backward) the onset of rotation resulted in significant suppression of phototaxic behavior at a 24 hr test interval for the simultaneous arrangement alone (median=.29; n=9). Forward and backward conditioning groups were not suppressed (.54, .42; n's=10,6 respectively).

Simulation of these training sequences in the isolated nervous systems of previously untrained animals by pairing light with depolarizing current stimulation of a caudal hair cell (cf. J. Farley and D.L. Alkon, J. Neurophysiol., 1983 in press) revealed a similar pattern for cumulative depolarization of Type B cells. These cells are causally related to phototaxic suppression (Farley et al., Science, 1983, in press). Five simultaneous pairings resulted in an average 7 ± 2 mV depolarization, which significantly exceeded that produced by forward (1.0 ± 1.7 mV; $p < .05$), backward (1.6 ± 2.0 mV; $p < .05$), and random control (2.0 ± 1.8 mV; $p < .05$) conditions. Consistent with previous results, the cumulative depolarization for the simultaneous arrangement arises in large part from increased positive synaptic feedback from the S/E optic ganglion cell and disinhibition from the caudal hair cell. Both are unique outcomes of terminating visual and hair cell stimulation simultaneously. For forward pairings, the asynchronous offsets of visual and statocyst stimulation results in post-light hair cell inhibition of the B cell, and substantially reduced and delayed positive synaptic feedback. For backward pairings the sole source of positive synaptic feedback is that attributable to light-alone.

268.PO EXTINCTION OF ASSOCIATIVE LEARNING IN HERMISSENDA: BEHAVIOR AND NEURAL CORRELATES William Richards, Joseph Farley, Dept. of Psychology, Princeton Univ., Princeton, NJ 08544 and Daniel L. Alkon, (spon: R. Cholewiak) NINCDS, NIH, MBL, Woods Hole, MA 02543

Long-term, pairing-, and light-specific reductions in Hermissenda phototaxic behavior have repeatedly been demonstrated for animals exposed to light-rotation pairings. The reversible nature of this form of associative learning is illustrated here by our findings that animals exposed to 25 non-reinforced light steps, immediately following the end of two daily sessions of standard light-rotation pairings (50/day), exhibited significantly reduced levels of phototaxic suppression (suppression ratio score: .44 ± .05), at a 2 hr retention interval, when compared to animals which received pairings only (.29 ± .05; $p < .05$). Extinction animals were no different from random control animals (.54 ± .05). Similar results were obtained at both 2 and 24 hr retention intervals in a second experiment, indicating a lack of spontaneous recovery of the extinguished phototaxic suppression. To test the possibility that habituation contributed to the extinction effect, an additional group of animals was exposed to the same total number (125) of light presentations as extinction animals, but were found to be unchanged by the experience (.56, .47; ± .05:2,24 hr. respectively). The absence of both habituation and sensitization of phototaxis, coupled with the failure to demonstrate spontaneous recovery, suggests that extinction in Hermissenda involves a reversal of the original neural substrates of acquisition and retention. This conclusion was supported by the results of intracellular recordings from isolated Type B photoreceptors from trained animals. These cells have previously been shown to be causally related to the learned suppression of phototaxis (Farley et al., Science, 1983 in press). Paired-condition animals exhibited significantly greater: 1) peak- (26.5 ± 1.4 vs. 19.9 ± 2.5 mV) and steady -state (20.3 ± 1.8 vs. 12.7 ± 2.3 mV) light-induced generator potentials, 2) greater input resistances (33.3 ± 8.9 vs. 25.2 ± 7.1MΩ) when compared to random control or naive animals. Extinction-animal light-responses (20.5 ± 1.5 mV, 15.3 ± 1.5 mV peak and steady-state) and input resistances (14.7 ± 2.5 MΩ) were no different from controls, but significantly less than paired animals ($p < .05$). Thus, Type B cells encode for extinction as well as acquisition and retention of associative learning. Additional results implicate primary neural changes to occur with acquisition and extinction training in Type A photoreceptors as well. Isolated Type A cell steady-state light responses were less for paired (14.4 ± 1.7 mV) vs. extinction (18.3 ± 2.5 mV) and naive or random control (17.2 ± 1.6 mV) animals.

HUMAN NEUROPSYCHOLOGY II

269.1 INTENSITY-TIME FUNCTIONS FOR PATTERN DISCRIMINATION IN RHESUS MONKEYS AND A HUMAN SUBJECT: EVIDENCE FOR SUPERSUMMATION. Andrew Glover*, Pedro Pasik and Tauba Pasik (Spon: Catherine Mytilineou), Dept. Neurol., Mount Sinai Sch. Med., CUNY, N.Y., N.Y. 10029.

Bloch's Law states that intensity (I) and exposure time (T) can be reciprocally interchanged below a certain critical duration (tc) to produce a constant visual behavioral response. This relationship is termed temporal summation. In general, for $T > tc$, I remains constant for the same effect. In addition, there is evidence suggesting that response latencies are similar below tc. Most studies on temporal summation have used single or double light flashes in human subjects. We sought to obtain this type of data using patterns in both normal monkeys and human observers.

Low contrast (12%) circle-triangle pairs were presented at several luminance levels, and subjects were required to initiate forced-choice trials. In Experiment I, tachistoscopic thresholds were obtained for two monkeys at two luminance values (2.9 and 0.2 cd/m^2), by both the descending staircase and the constant stimuli methods. Stimulus and adapting fields were flux-equated for each level. Thresholds (means of 14 and 34 msec, respectively) were found comparable across procedures. The 1.2 log attenuation of the luminance resulted in only a 0.4 log increase in liminal duration. Similarly, less energy (E = IT) was needed at the lower luminance value, the difference being 0.8 log Talbot.

In Experiment II, thresholds were determined in three monkeys and one human subject by the descending staircase method. Eight luminances were used in 0.3 log unit steps. Contrary to Experiment I, the adapting field was held constant and flux-equated to the highest stimulus value (2.9 cd/m^2). For the same monkeys used in Experiment I, a 1.2 log decrease in luminance resulted in about 1.0 log increase in threshold duration, the energy difference being approximately 0.3 log Talbot. For all subjects, function analyses of the log I vs log T relationships showed a region of reciprocity (slope = -1.0), in agreement with Bloch's Law, for brief durations. However, at longer exposures the slopes were not of the expected 0.0 value (failure of summation) and, therefore, no tc was evident. Instead, the slopes became steeper than -1.0, i.e., T was more effective than I for eliciting the criterion response. Latencies remained relatively constant for high luminance, brief duration stimuli, and became progressively longer at lower luminances and longer durations.

The results of both experiments indicate that, at longer exposures, T is a more significant stimulus property than I for a pattern discrimination task. This effect has been termed supersummation. The inflexion point between reciprocity and supersummation may serve as a marker for separating different processes used to solve similar visual tasks. The phenomenon has been only rarely found in physiological and behavioral studies probably because no complex stimuli (patterns) were used. Apparently, light adaptation influences the magnitude of the supersummation effect, given the differences found across the present experiments. (Aided by NEI #EY-07014 and EY-01867.)

269.2 BRAIN ORGANIZATION EXPLAINS DUAL-TASK INTERFERENCE: A DEVELOPMENTAL STUDY. M. Kinsbourne* M. Hiscock* (SPON. J. Warsh). Eunice Kennedy Shriver Center, Waltham, MA. 02254

We have attributed the detrimental effect of performing two tasks at the same time ("time-sharing")to the need to engage a mechanism that obviates interfering neuronal activity ("cross-talk") between the concurrently active cerebral processes. If so,the time sharing decrement should be a function of the extent to which the relevant areas of central cortex are connected or customarily interact. Specifically, it should be greater in, but not limited to, the situation in which both tasks are programmed within the same hemisphere. Should cross-talk be incompletely inhibited, this should manifest as intermittency in task performance. This should be more apt to happen in the immature brain.

Seventy-three righthanded children in grades one through four engaged in speeded finger tapping of the right and of the left hand, each while 1. speaking, 2. memorizing, 3 not otherwise performing. Both concurrent tasks reduced tapping rate relative to control 1. in proportion to task difficulty, 2. of the right hand more than the left, 3. in inverse proportion to age. Gross intermittency characterized the time shared tapping of grade one children only. Ability to time share increased with age, but the degree to which interference was lateralized did not.

The results indicate greater ability to isolate active central processors from each other when they are located indifferent hemispheres and when the brain is relatively mature. Time sharing engages neural activity additional to that which controls the concurrent performances. This activity becomes more efficient with increasing age.

The results do not readily lend themselves to interpretation in terms of unitary or multiple resource models of capacity limitation.

269.3 ENHANCED DUAL TASK PERFORMANCE FOLLOWING TRANSECTION OF THE CORPUS CALLOSUM IN HUMANS. J. D. Holtzman* and M. S. Gazzaniga. Div. of Cognitive Neuroscience, Cornell Univ. Med. Col., N.Y., N.Y. 10021.

Surgical transection of the corpus callosum in humans, so-called "split-brain" surgery, has profound effects on the perception of the visual environment. In essence, when the accurate performance of a task requires the detailed analysis of visual pattern, callosal surgery renders each hemisphere perceptually blind to visual stimuli in the ipsilateral hemifield, i.e., the right hemisphere only perceives left field stimuli, the left hemisphere only perceives right field stimuli. As a consequence, split-brain patients are unable to perceptually compare stimuli appearing in different hemifields. Based on these findings, we hypothesized that under conditions of maximal perceptual load, in which stimuli are distributed between the hemispheres, the apprehension of visual stimuli by split-brain patients would be superior to that of neurologically intact observers.

The present findings confirmed this prediction in one split-brain patient at a spatial memory task. The background visual display for these experiments consisted of two 3 X 3 cell matrices, one displayed to the left and one to the right of a central fixation stimulus. On each trial, a bilaterally displayed "X" moved among 4 cells, either through the same or a different sequence of cells in the two matrices. A unilateral probe sequence subsequently appeared, and the observer indicated whether it matched the original sequence for the probed field. The split-brain patient's performance was comparable under the two conditions ($z = .69$; $p > .05$), whereas neurologically intact observers were significantly more accurate when the same pattern appeared in the two hemifields ($z = 9.42$; $p < .001$). Moreover, the split-brain patient performed better than the control observers when different sequences were presented in the two matrices ($z = 4.70$; $p < .001$). This latter finding implies that under experimental conditions that maximize interfield perceptual load, surgical transection of the corpus callosum can enhance the information processing capacity of the organism.

Supported by NIH grants R01 NS 17936 and P01 NS 17778.

269.4 OPPOSITE HEMISPHERE LATERALIZATION OF EXPRESSIVE AND RECEPTIVE SPEECH MECHANISMS: AN UNUSUAL CASE STUDY OF CROSSED DOMINANCE. D. Buchholz,* D. Ratusnik,* L. Bieliauskas,* and F. Morrell. Departments of Psychology, Speech Pathology and Neurological Sciences, Rush Medical College, Chicago, Il. 60612.

Early brain injury to the dominant hemisphere frequently results in a shift of speech dominance to the opposite side. Most commonly such shift involves all aspects of language processing. It has been suggested, however, that the shift may be selective for one or another facet of speech (Rasmussen and Milner, Ann. N.Y. Acad. Sci. 299: 355, 1977) although such selectivity has rarely been documented by pre and post-operative examination. We present a study of a 33 yr old left handed accountant who had sustained a perinatal left hemisphere injury resulting in chronic epilepsy. A large porencephalic cyst replaced much of the posterior inferior parietal and posterior temporal lobe on the left. When the seizures became medically intractible, he was hospitalized for evaluation for surgical therapy.

Intracarotid amytal injection into the left carotid artery resulted in complete cessation of ongoing speech, despite retained comprehension of verbal commands. The patient also recalled verbal material given during the drug effect including names of objects and a test phrase. Right carotid injection of amytal did not result in cessation of counting or other serial verbal tasks. Verbal production was fluent but aphasic and he could not follow commands given during the drug action. The test phrase was not recalled.

Pre and post-operative neurological, neuropsychological tests as well as the Boston Diagnostic Aphasia Examination confirmed the interpretation of the amytal studies.

This case offers additional support to the notion of Rasmussen and Milner (1977) that brain injured patients may have receptive speech function in one hemisphere and expressive speech in the other.

269.5 THE CORPUS CALLOSUM IS LARGER IN LEFT HANDERS. S. F. Witelson. Dept. Psychiatry, McMaster Univ. Hamilton, Canada, L8N 3Z5

Linear and planimetric measurements of the corpus callosum exposed in midsagittal section were made in 33 consecutively obtained formalin-fixed specimens acquired from clinical post-mortems. They included 12 men and 21 women, ranging in age from 25 to 67 years (mean = 49 yr) at time of death. The direction, degree and family history of hand preference were available from direct testing of these cases who are being studied as part of a larger study on the relationship between neuroanatomical variation and neuropsychological test performance. All anatomical measurements were made without the knowledge of handedness or sex of the individual.

It was found that individuals with left- or mixed-hand preference (n=11) (as defined in Annett, Quart. J. exp. Psychol, 1967, 19, 327) had a larger corpus callosum than did right handers (n=22). The two hand subgroups, by chance, proved comparable in age, sex distribution and brain weight. Mean total area of the callosum was 6.7 vs 7.9 cm^2 for the right and left handers, respectively. Mean brain weight was similar for right and left handers, 1311 vs 1359 g, respectively. Callosal area relative to brain weight (x1000) was also significantly different between groups, 5.1 vs 5.8, respectively ($p<.01$). This difference between hand groups was present within each sex group, particularly for men. Maximal antero-posterior callosal length was 7.1 vs 7.4 cm for right and left handers, respectively (nonsignificant).

Callosum size was found also to be associated with sex, as previously reported (de Lacoste-Utamsing & Holloway, Science, 1981, 216, 1431). In the present study, the sexes were found to differ, not in callosal area (7.2 vs 7.0 cm^2), callosal length (7.3 vs 7.1 cm) or maximal splenial width (1.5 vs 1.4 cm), but only in callosal area relative to brain weight x1000 (5.0 vs 5.6, $p<.05$); in all cases, men vs women, respectively. Rank order of callosal area relative to brain weight in the four subgroups indicates that right-handed men have the smallest corpus callosum (4.6) and left-handed women, the largest (5.9).

Hemispheric representation of cognitive functions is well documented to be more diffusely represented bilaterally in left handers compared to right handers; some evidence indicates that females also have greater bihemispheric representation of cognition compared to men (Witelson, In Language Functions and Brain Organization, Segalowitz (ed) 1983). The larger corpus callosum found in left handers and also in females may be a morphological basis for individual differences in laterality and for the greater bihemispheric representation of cognition in some individuals.

NIH-NINCDS Contract N01-NS-6-2344 and Grant R01-NS18954.

269.6 NON-HEMORRHAGIC LESIONS OF THE THALAMUS: NEUROPSYCHOLOGICAL, NEURORADIOLOGICAL AND ELECTROPHYSIOLOGICAL FINDINGS IN HUMANS. Authors: Neill R. Graff-Radford*, Hanna Damasio*, and Thoru Yamada*, (SPON: A.E. Applebaum). Department of Neurology, University of Iowa Hospitals, Iowa City, Iowa 52242

Eighteen patients with non-hemorrhagic thalamic infarctions were independently studied by three investigators with: (a) comprehensive clinical and neuropsychological testing (NG-R); (b) computerized tomography (CT) (HD), and (c) somatosensory evoked responses (SER) (TY). The patients were divided into five groups corresponding to the probable vascular territories of the infarctions. These include (i) entire geniculothalamic territory (n = 4), (ii) partial geniculothalamic territory (n = 3), (iii) tuberothalamic (n = 3), (iv) deep interpeduncular profunda (n = 2), (v) anterior choroidal (n = 6).

Severe neuropsychological deficits were seen in the tuberothalamic and deep interpeduncular profunda groups. Language, verbal and visual memory, verbal and performance IQ and visuospatial deficits were seen in the left tuberothalamic patients, whereas language, verbal memory and verbal IQ were preserved with right tuberothalamic lesions. Left anterior choroidal cases had language and verbal memory deficits, whereas the right anterior choroidal had visual memory deficits. The geniculothalamic groups had normal neuropsychological performance unless the posterior cerebral artery was also occluded.

SER's were absent after P14 (brain stem) over the ipsilateral hemisphere in the complete geniculothalamic artery group, possibly corresponding to destruction of the primary sensory nuclei (VPL and VPM) and to the clinical finding of multimodal sensory loss. In the partial geniculothalamic group pinprick loss and proprioception retention were associated with normal N17, N19 and N20 but delayed N32 and N60. This indicated that the stimulation of medial lemniscal pathways probably caused waves N17, N19 and N20. But lesions in the tuberothalamic territory, which does not include the primary sensory nuclei, cause a delay of these waves. This may result from a disturbance of intrathalamic pathways or disturbance of the gating by the reticular nucleus of the thalamus situated anterior to VA. This area is considered important in gating evoked responses in the cat.

269.7 COGNITIVE DEFICITS ASSOCIATED WITH RIGHT HEMISPHERE DAMAGE: COMMON PROCESSES UNDERLYING VISUOSPATIAL ABILITIES AND STORY COMPREHENSION? Kenneth L. Moya*, Larry I. Benowitz, David M. Levine* and Seth Finklestein*. Mailman Research Center, McLean Hospital; Depts. of Psychiatry and Neurology, Harvard Medical School; Massachusetts Rehabilitation Hospital; Massachusetts General Hospital.

Visuospatial abilities and story comprehension were tested in 18 right brain-damaged (RBD) patients and in 10 age-matched controls. Performance for both the story comprehension and visuospatial tasks was evaluated for recall of details and for appreciation of overall form and relationships among elements, using a quantitative scoring protocol developed for these studies. In addition, the frequency of various error types such as perseverations, intrusions, substitutions, and confabulations were analyzed. In accordance with other research, our results showed that RBD patients were impaired on all aspects of visuospatial performance. RBD patients also performed significantly worse on story comprehension than controls, and these deficits were correlated with the degree of visuospatial deficits. The ability to appreciate relationships among story elements showed the greatest impairment after right brain damage and was the most significant verbal correlate of visuospatial performance. These results indicate (a) that certain aspects of language transcend the capabilities of the isolated left hemisphere, and (b) that the involvement of the right hemisphere in appreciating complex linguistic material may utilize processes similar to those involved in visuospatial abilities. We propose that both story comprehension and visuospatial abilities reflect a general role for the right hemisphere in organizing and/or evaluating schemata. Supported by an Alfred P. Sloan Foundation Fellowship to Dr. Benowitz, and an American Heart Association Clinician-Scientist Award to Dr. Finklestein.

269.8 NEUROPSYCHOLOGY OF MILD HEAD INJURY. H.M. Eisenberg, H.S. Levin, R.M. Ruff,* S. Mattis,* L.F. Marshall and K. Tabaddor.* Div. of Neurosurgery, Univ. Texas Med. Branch, Galveston, TX 77550; Div. of Neurosurgery, Univ. Calif., San Diego, and Div. of Neurosurgery, Albert Einstein College of Medicine, Bronx, NY.

In an ongoing three-center study, we have investigated the recovery of 76 consecutively admitted patients with mild closed head injury (CHI). The head injuries were considered mild according to research diagnostic criteria; no other major injuries were present. The neuropsychological measures consisted of the Mattis-Kovner selective reminding test, the Benton Visual Retention Test, Digit Span, a visuomotor speed test (Coding), and the Paced Auditory Serial Addition Test (PASAT).

In comparison with a control group (n=52), the mild CHI patients were impaired on all measures at the time of the baseline exam within 1 week of injury. According to a criterion of the worst control score, nearly 50% of the mild CHI patients had defective verbal memory, a third had poor visual memory and about 25% evidenced deficits on digit span, Coding, and the PASAT.

Thirty one mild CHI patients have been examined at 1 month postinjury. Their performance improved on all measures ($p<.001$) and showed only a nonsignificant trend of deficit as compared to the controls. Review of individual cases and each test, however, disclosed that 10% to 30% of the mild CHI patients were still impaired.

269.9 AUTOMATIC AND EFFORTFUL PROCESSING IN DIABETICS. W. Lichty. Psychology Department, University of Missouri, Columbia, MO 65211

Two aspects of information processing, effortful and automatic processing, were investigated in 32 Type I (insulin-dependent, ketone-prone) diabetics and 32 control subjects matched for age, sex, and education. Effortful processing efficiency varies with organismic and environmental influences such as aging, mood, and stress; whereas, efficiency for automatically processed information is relatively constant. The purpose of the present experiment was to investigate effortful and automatic processing in diabetes, a disease which is subject to neurological influences involving acute effects of blood glucose level and chronic effects related to cardiovascular complications and to segmental demylination of Schwann cells, and in some cases, oligodendrites.

As anticipated, on a task requiring effortful processing, a paired-associate learning task, diabetics attained lower scores when WAIS vocabulary scores were partialed out in an analysis of covariance ($p<.05$). On the automatic processing task, a frequency judgment task, null effects were found ($p=.61$), in agreement with the hypothesis that automatic processes are stable under physiological alterations.

Additional investigations of the diabetic group revealed possible effects immediate blood glucose level and long term diabetic control may have on effortful processing. Correlations of blood glucose level and paired-associate learning scores increased over trials and eventually attained significance, thus suggesting a positive influence high glucose availability in the brain may have on effortful processing. Diabetic control may also have a strong impact on effortful processing, as suggested by superior performance of subjects who reported high frequency of hypoglycemic reactions ($p<.05$). Since frequency of hypoglycemic reactions is a rough indication of general level of diabetic control and since general level of control relates to development of complications, it is possible that the results relate to neurological degeneration.

269.10 DISPROPORTIONATE DECLINE IN VISUOSPATIAL MEMORY IN HUMAN AGING, H.S. Levin and G.J. Larrabee*. Div. of Neurosurgery and Dept. of Neurology, Univ. Texas Med. Branch, Galveston, TX 77550.

In view of the frequent finding of memory deficit and visuospatial impairment early in the course of dementia of the Alzheimer type (cf. Sim, M. & Sussman, I. J. Nerv. Ment. Dis., 135:489, 1962), we postulated that long term memory (LTM) declines more rapidly as a function of age for visuospatial information as compared to verbal material. We employed the selective reminding test (Buschke, H. & Fuld, P.A. Neurology, 24:1019, 1974), which measures storage and retrieval of 12 words across 12 trials. We also administered a visuospatial selective reminding task in which the subject reproduces the spatial locations of marbles over 12 trials. Consistent long term retrieval (CLTR) summed across the 12 trials was the measure of LTM. We studied 50 high school students, 22 subjects in the 60-69 year old range, 24 subjects in the 70-79 year old range, and 17 subjects at least 80 years old (mean =83) who had adequate vision and hearing and no history of major health problems.

We transformed the CLTR scores of the elderly subjects to standard (T) scores based on the CLTR distribution of the high school students (T score mean = 50, SD = 10). The T transformation of CLTR scores on the verbal and visuospatial selective reminding tasks in the 60-69 year old group yielded means of 39.7 (SD=13.5) and 19.2 (SD=27.7), respectively which differed significantly ($t=3.13$, $p<.005$). In the 70-79 year old group visuospatial memory (mean=5.4, SD=33.4) was significantly below verbal memory (35.0, SD=9.1), $t=4.14$, $p<.001$. The pattern of more rapid decline in visuospatial memory was also confirmed in the subjects who are at least 80 years old (verbal CLTR=28.9, SD=10.9; visuospatial CLTR=1.41, SD=27.5), $t=3.73$, $p<.002$.

Impairment of CLTR, i.e., a score which fell at or below the 5th percentile of the students, was present in 8 of the 60-69 year olds (36%) on the spatial selective reminding as compared to 4 (18%) failures on the verbal procedure, a nonsignicant difference. In the 70-79 year old group, 9 subjects (38%) were impaired on the spatial task as compared to only a single subject (4%) on the verbal measure, a difference in proportions which was significant ($\chi^2=6.19$, $p<.02$). Of the subjects at least 80 years old, 5 (29%) had impaired visuospatial memory as compared to 2 (12%) with verbal LTM deficits, a nonsignificant difference.

We suggest that learning and retention of visuospatial information is more vulnerable to the effects of normal aging than memory of verbal material. The disproportionate decline in visuospatial memory in normal aging may represent an analogue of the memory disorder associated with dementia of the Alzheimer type.

269.11 COMPUTED TOMOGRAPHY MEASURES OF CEREBRAL ATROPHY AND COGNITIVE DECLINE IN AGING AND DEMENTIA: T. L. Jernigan and L. M. Zatz*. Veterans Administration Medical Center, Palo Alto, CA 94304.

We have obtained measures from computed tomography (CT) of sulcal widening, ventricular enlargement, and mean attenuation values in white and gray matter samples in over 175 normal adults and over 40 demented patients. In two independent studies of older adults, using different CT scanners, white and gray matter values declined with age. This change appears to be more strongly associated with sulcal widening than with ventricular enlargement, and occurs quite evenly across the different structures sampled. Like the global atrophy measures, the white and gray matter values are modestly, but significantly correlated with measures of recent and remote memory, and psycho-motor speed.

In our investigations of the normal elderly, specific relations between regional CT measures and various cognitive test scores have been only weakly supported. For example, we hypothesized that frontal white matter values would be more strongly associated with psychomotor speed while temporal white matter values would better predict performance on a figure learning task. The simple correlations were as follows:

	Frontal White	Temporal White
Psychomotor Speed	.28*	.20
Figure Learning	.28*	.42**

While the results are not inconsistent with our hypotheses, regression analyses reveal that the difference between the contributions of the CT values does not approach significance for either cognitive measure. That is, no specific relationship is observed between frontal white and psychomotor speed, or temporal white and figure learning.

With CT, we can not yet rule out a model in which parenchymal values decrease and sulcal widening occurs diffusely due to a single global process. This process would appear to be weakly associated with pervasive mild intellectual decline. Analyses of data from demented patients are currently in progress. These results will be compared to those obtained in normal aging.

*p<.05, **p<.01.

269.12 NEUROPSYCHOLOGICAL FUNCTION AND REGIONAL CEREBRAL GLUCOSE UTILIZATION IN HEALTHY AGING AND DEMENTIA. C.L. Grady*, J.V. Haxby*, R. Duara*, S.I. Rapoport and N.R. Cutler. (SPON: A.F. Mirsky), NIH/NIA, Laboratory of Neurosciences, Bethesda, MD, 20205.

To assess the relation between behavioral and physiological measurements of brain function, we have compared performance on the Luria Nebraska Neuropsychological Battery (LNNB) with regional cerebral metabolism, as measured by [^{18}F]fluoro-deoxyglucose and positron emission tomography (PET). Thirty-seven healthy male subjects between the ages of 21-83 yr (mean age 49.9) were PET scanned with reduced visual and auditory input. The regional cerebral metabolic rates for glucose (rCMRglc) and ratios of rCMRglc to whole brain CMRglc (Q scores) were calculated and correlated with age and the clinical scales of the LNNB. The LNNB scores also were arranged into localization scales, which are thought to represent function of large brain areas, such as the frontal lobes, and the rCMRglc values and Q scores were also grouped into corresponding regions. These measures were then correlated with age and with each other. None of the rCMRglc values or Q scores was correlated with age but the clinical scales of the LNNB, with the exception of reading, writing, and arithmetic, were significantly correlated with age. Few rCMRglc values or Q scores were correlated significantly with any LNNB scale, and these could have been due to chance. The localization scales were significantly correlated with age, with the exception of the left parieto-occipital scale, while none of the combined metabolic values was related to age. Only one localization scale was significantly correlated with the corresponding metabolic value, that of the left parieto-occipital scale with the corresponding Q score.

A similar analysis was conducted on the data from 4 patients with a diagnosis of Alzheimer's disease (mean age 57.7 yr) who were also PET scanned in an unstimulated state. In 3 patients there was close correspondence between abnormally elevated LNNB localization scales and metabolic deficits, particularly in the parieto-occipital areas. A fourth patient showed a marked right parietal deficit on the LNNB and a predominantly left parietal metabolic deficit.

The findings suggest that in healthy subjects there is little relation between neuropsychological function as measured by the LNNB, and rCMRglc in an unstimulated state. In patients with dementia, however, there is a correspondence between these two measures, particularly in the parieto-occipital areas, suggesting that both instruments may be useful in assessing gross deficits in brain function.

269.13 RETENTION OF ORIENTING REACTION HABITUATION IN PATIENTS WITH NIGHT TERRORS. R. Rogozea and V. Florea-Ciocoiu+. Inst. Neurol. Psychiat., Bucharest, ROMANIA.

A polygraphic study on habituation of the somatic (EMG), autonomic (finger plethysmogram, galvanic skin reaction, pulse, EKG) and EEG (acoustic-evoked potential and EEG-blocking reaction) components of the orienting reaction elicited by a repetitive auditory stimulus during successive (weekly) sessions was performed in 36 patients with night terrors and in 72 matched subjects in two control groups (41 normal subjects and 31 patients with symptomatic epilepsy).

The study evidenced significant retention disturbances of orienting reaction habituation (i.e. of learning), the "saving" of stimulations achived from one session to the other to obtain the habituation criterion, being lesser in patients with night terrors than in normal subjects of control group I. However, the "saving" of stimulations was significantly bigger than in patients with symptomatic epilepsy of control group II.

The severity of these habituation retention disturbances in patients with night terrors depended on the patients' age at seizure onset, the frequency of nocturnal episodes and their etiology, as well as on the features of spontaneous EEG activity. Thus, the most marked deficit in retention of the orienting reaction habituation was noted in patients with early onset of the nocturnal episodes (i.e. undes the age of 9), in patients with frequent seizures (i.e. with daily seizures), in patients with nocturnal episodes on organic background (i.e. with meningoencephalitides, craniocerebral traumata, neonatal diseases in their history) and in patients with interictal EEG tracings displaying signs of hyperexcitability, diffuse or focal pathological graphoelements and especially EEG signs of immaturity.

These habituation retention disturbances may be ascribed to the nervous functional disordes induced by psychological factors (mental stress, strong emotional states, environmental (sociofamilial) negative stimuli) or/and by organic disordes (caused by post-meningoencephalitic or post-traumatic lesions) which determine, apart from night terrors seizures, disturbances in the activity of the neural structure and pathways involved in the regulation of the nervous system diffuse excitability and, implicitly, in the control of repetitive sensory messages and in learning.

269.14 TOWARD A THEORETICAL NEUROPSYCHOLOGY: BRAIN SYSTEMS AS MICROFIBRATIONS. W. C. Hoffman. Dept. of Mathematical Sciences, Oakland University, Rochester, MI 48063.

The topological structure of microfibration (a "total space" coupled to and from a "base space" by projection mappings) appears to be the common structural and functional element of the CNS. In the case of the posterior perceptual systems, these fibrations constitute so-called equivariant dynamical systems, owing to the known invariances of form perception. For such cognitive functions as semantic memory and problem solving, the fibrations consist, according to information processing psychology, of semisimplicial microbundles embodied in the structure of the prefrontal cortex, inferotemporal cortex-hippocampus, and the higher brain stem. The latter constitutes the base space for subcortical integration of higher brain functions at the conscious level. Specific features of LTM, STM, and working memory and of problem solving behavior are analyzed in terms of the structure of the category of semisimple fibrations.

270.1 HAIR FOLLICLE INPUTS CONVEYED TO THE FIRST SOMATIC SENSORY AREA (SI) VIA THE DORSAL FUNICULUS ARE REQUIRED FOR THE PERFORMANCE OF A HAIR DISPLACEMENT DISCRIMINATION BY RHESUS MONKEYS.
Richard J. Schneider and David P. Friedman. Laboratory of Neuropsychology, Bldg 9 Rm 1N107, NIMH, Bethesda, MD 20205.

The role of the dorsal funicular input to SI in hair displacement and electrocutaneous stimulus discrimination was explored. Three rhesus monkeys were trained to discriminate between two amplitudes of displacement of hair groups on the anterior calf and between two frequencies of electrical pulse trains applied to the skin over the peroneal or sural nerves. A go-no go design was employed. Each animal was required to press a manipulandum in response to the go stimulus (a specific amplitude of hair displacement or frequency of pulse train) and to refrain from pressing it for the no-go stimulus (a lower amplitude of hair displacement or frequency of pulse train). The discriminations were made ambiguous so that each animal made errors while performing the task. The stimuli were presented successively (10 sec. interstimulus interval) and pseudorandomly. After reliable control values were established, animals were retested with peripheral nerve block (PNB) followed by unilateral dorsal funiculus (DF) lesions at the T1 level. Following the DF lesions and testing, single and multiunit recording in SI was carried out in one animal. Contralateral to the lesion, all of the denervated and some of the innervated portions of SI were explored. In the opposite SI, similar regions were examined.

Prior to the tractotomy, discriminative ability of monkeys on the hair displacement and electrocutaneous tasks produced mean d' scores, the measure of sensory capacity in signal detection theory, of 2.21 and 1.29, respectively. With PNB, responding to both types of stimuli ceased distal to the block, but not proximal to it. By contrast, following DF tractotomy, there was a marked diminution in the d' score below the level of the lesion on the hair displacement but not on the electrocutaneous task. Contralateral to both the PNB and DF tractotomy, there was no change in d' scores. In the region of SI deprived of DF input (lower body representation), no cells were found which responded to hair displacement and none was driven electrocutaneously. Both stimuli drove cells in the arm representation on the ipsilateral side and the leg representation contralaterally.

The results suggest that: (1) SI is supplied with hair displacement input exclusively via the DF; (2) Hair receptor input to SI is required for hair displacement discrimination; and (3) Electrocutaneous input to SI also arrives exclusively via the DF but this input is not required for the discrimination of pulse train frequency.

270.2 GLUTAMIC ACID DECARBOXYLASE CONTAINING NEURONS IN THE DORSAL COLUMN NUCLEI OF THE CAT. A. Rustioni, D.E. Schmechel*, S. Cheema., and D. Fitzpatrick,* Depts of Anatomy and Physiology, Univ. of NC at Chapel Hill, and Dept. of Neurology, Duke Univ. Durham, NC

The retrograde transport of horseradish peroxidase (HRP) and immunocytochemistry for glutamic acid decarboxylase (GAD) have been employed to verify whether interneurons exist in the dorsal column nuclei and whether these may be GABA-ergic. Observations were focused on the dorsal part of the middle cuneate nucleus (MCd) since this region has been previously shown to contain projecting neurons whose axon terminate almost exclusively in the contralateral thalamus. After injections of HRP or lectin conjugated HRP (WGA-HRP) in the ventrobasal complex of the feline thalamus the majority of neurons in MCd are labelled. These represent 88.9%, as counted in frozen, 40 µm thick sections, and 69.7% as counted in plastic-embedded 2.5% µm thick sections, of the neuronal population of MCd. Unlabelled by the same injection are few medium to large neurons at the dorsal rim of MCd but, most characteristically, small (X=±250 µm^2) neurons at the periphery of the cell clusters formed by thalamic-projecting neurons. These small neurons rep-resent 10-12%, as counted in frozen, 40 µm thick sections, and 29.3%, as counted in plastic-embedded 2.5 µm thick sections, of the neuronal population of MCd. The same class of neurons are also unlabelled after injection of the retrograde tracer in the pretectal area, the inferior and superior colliculi, the inferior olivary complex and/or spinal cord. These injections however re-sult in labelling of neurons along the dorsal rim of MCd and/or in other regions of the cuneate nucleus.

In adult, colchicine treated, cats, the use of anti-GAD-serum reveals a population of labelled neurons uniformly distributed throughout the DCN. In MCd, these are small (X = 220µm) neurons found mainly at the periphery of cell clusters and they represent about 27% of the neuronal population of this nuclear subdivision as counted in plastic-embedded 2.5 µm thick sections. Labelled processes densely infiltrate the cell clusters and labelled varicosities appear to cover the soma and dendrites of unlabelled neurons.

At the electron microscopical level, most labelled profiles contain vesicles and correspond to F-boutons usually involved in "axo-axonic" contacts with terminals of dorsal root afferent and presynaptic to dendrites. Other vesicle-containing, GAD-positive endings also seem to correspond to the P-boutons described by Ellis and Rustioni (1981) and are believed to be, at least in part, of dendritic origin. It is suggested that GAD-positive neurons are GABA-ergic interneurons and that these can mediate both pre- and post-synaptic inhibition although their integrative role is likely to be more complex than postulated by previous electrophysiological studies. Supported by USPHS grant NS 12440.

270.3 CLASSIFICATION OF SLOWLY-ADAPTING PROPRIOCEPTIVE NEURONS IN THE CUNEATE NUCLEUS OF THE CAT. D.J. Surmeier*and A.L. Towe. Dept. of Physiology and Biophysics, Univ. of Washington, Seattle, Wa.98195

Muscle spindle afferent fibers from the forelimb project to the cuneate nucleus of cats (Rosen,J.Physiol.,205:209-236,1969). Amassian and Giblin (J.Physiol.,243:353-385,1974) reported that proprioceptive cuneate neurons rarely produced spike trains with more than two periodic components, and suggested that such neurons are strongly driven by no more than two periodically discharging muscle afferent fibers. We examined this assertion by analyzing the spike trains of cuneate neurons with both time and frequency domain methods. In addition, we attempted to characterize the cutaneous receptive fields of these cells.

Single unit activity was recorded with tungsten microelectrodes from domestic cats anesthetized with alpha-chloralose and paralyzed with decamethonium bromide. Spike and stimulus trains were led into waveform discriminators interfaced with a TI 990/12 computer that recorded event times on magnetic disk. All units had slowly-adapting responses to alterations in forelimb position, and could not be driven by gentle probing or stretching of the skin. The spike train produced in the absence of cutaneous stimulation was recorded with the limb held fixed. Then, electrical stimuli were delivered to the skin through needle bipolar electrodes. The stimuli were Poisson distributed, with mean rates of 2.5-10 Hz. Responses were assessed by cross-correlation.

Three types of proprioceptive neuron were found. The most common type (26/56) had unimodal interval and joint interval distributions and one, or a few, closely spaced spectral components. Only one of twelve units tested responded to cutaneous stimulation. The second type (11/56) had bimodal interval distributions and joint interval structures indicative of two or more strong periodic inputs. Spectral analysis revealed from two to five periodic components. Two of four cells tested had cutaneous receptive fields. The third type (19/56) produced spike trains which "reset" following a non-modal interval. These trains rarely had fewer than three dominant spectral components, and commonly had four or five. Of eleven cells tested, ten responded to cutaneous stimulation. Thus, while all proprioceptive neurons sampled displayed periodicities, the majority had one, or two, dominant components. A significant number of cells appeared to receive input from more than two muscle afferent fibers and from afferent fibers of cutaneous origin.

This work was supported in part by NIH grant NS 5136

270.4 DIFFERENCES IN THALAMIC- AND SPINAL-PROJECTING NEURONS IN CAT DORSAL COLUMN NUCLEI. R. J. Budell* and K. J. Berkley (SPON: J. E. Tunkl). Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306.

In addition to ascending projections, neurons in the dorsal column nuclei (DCN) have descending projections to the spinal cord (Dart, 1971; Kuypers & Maisky, 1975; Burton & Loewy, 1977). Recent electrophysiological studies have demonstrated that 70% of the spinal-projecting DCN neurons have ascending collaterals (Bromberg et al., 1981). The purpose of the present study was to determine, anatomically, if these ascending collaterals terminate in the ventroposterolateral nucleus of the thalamus (VPL).

Spinal- and VPL-projecting neurons were compared using single and double autoradiographic or fluorescent retrograde labeling techniques. The labels which were injected into the rostral spinal cord (C2, C4-8, T4) and VPL included: ^{3}H-inactivated horseradish peroxidase, ^{3}H-N-acetyl wheatgerm agglutinin and wheatgerm agglutinin:horseradish peroxidase conjugate, or the fluorescent dyes fast blue and nuclear yellow. In comparisons of the labeling patterns in 16 cats, spinal-projecting neurons differed in both their morphology and location from VPL-projecting neurons. Whereas, as expected from previous studies, round "clustered" neurons were labeled after injections confined to VPL, the majority of labeled neurons following spinal injections were fusiform, triangular or stellate. Furthermore, VPL-projecting neurons were grouped, as expected, into "cell nests" in the dorsal portion of the caudal and middle levels of DCN, whereas spinal-projecting neurons were concentrated ventrally in the same levels, as well as in distinct regions between the gracile and cuneate nuclei. At rostral levels of DCN, where VPL-projecting neurons were more scattered, spinal-projecting neurons remained ventral or ventromedial to the VPL-projecting neurons.

These results indicate that ascending collaterals of spinal-projecting DCN neurons probably do not terminate in VPL, but instead terminate in one or more of DCN's other targets. Because of their location and morphology, it is likely that this target, or targets, may be the pretectum, as well as perhaps the pons, cerebellum or tectum (Bull & Berkley, 1981; May & Berkley, this vol.).

The ventral portion of DCN receives sensory input from muscles and other deep structures, whereas the region between the gracile and cuneate nuclei receives input from thoracic segments (Rosén, 1969; Keller & Hand, 1970; Bakker et al., 1982). The present results together with previously reported results on pretectal- and pontine-projecting neurons (see above) suggest that the spinal-projecting neurons may be part of an efferent system originating in DCN that is involved in some aspect of sensorimotor integration such as the control of postural adjustments.

Supported by PHS grant RO1-NS-11892 from NINCDS.

270.5 A COMPARATIVE STUDY OF THE THALAMIC DISTRIBUTION OF GAD IMMUNOREACTIVE NEURONS. G.R. Penny, M. Conley, I.T. Diamond and D.E. Schmechel*. Neurobiology Program and Department of Psychology, and Division of Neurology, Department of Medicine, Duke University, Durham, NC 27706.

In earlier papers we described the distribution of neurons immunoreactive for glutamic acid decarboxylase (GAD) in the dorsal thalamus of the cat and the bushbaby, Galago senegalensis. The significance of these experiments relies on the fact that GAD is the synthetic enzyme for GABA and therefore that GAD immunoreactivity is a marker for inhibitory GABAergic neurons. One striking result was the similarity between these two distantly related groups. In both species the small GAD neurons were distributed throughout the dorsal thalamus with the exception of the centre median-parafascicular complex, where GAD neurons were absent. In both cat and Galago 20-30% of the neurons in the lateral geniculate and ventral posterior nuclei were GAD immunoreactive, and in both species the GAD neurons were identified as local circuit neurons (i.e., they did not project to cortex). In the present study we examined two additional species (opossum and rabbit), chosen because they might reveal some basic principles of the organization of thalamus in all mammals: Specifically, we asked, "are the similarities between cat and Galago common to all mammals or, on the contrary, does cat and Galago each reflect derived features differing from those displayed by the living conservative mammals (such as opossum)?"

The answer to this question was immediately apparent from the finding that in opossum, GAD neurons are chiefly confined to the lateral geniculate and the lateral extremity of the lateral posterior nucleus. The distribution of GAD neurons in rabbit is curiously intermediate between that found in opossum on the one hand and cat and Galago on the other. Like opossum, about 25% of cells in the lateral geniculate nucleus are GAD immunoreactive. Unlike opossum, however, as many as 18% of the cells in the ventral posterior nucleus are GAD immunoreactive, and scattered cells are also labeled in other thalamic areas, such as the medial geniculate and the lateral group. These results support the idea first put forward by Cajal that local circuit neurons proliferate with the course of evolution of complex mammalian brains. If we can assume that the opossum retains characteristics reflecting an early (or primitive) stage of mammalian evolution, the results suggest that thalamic local circuit neurons evolved first in the visual thalamus and only later in evolution spread throughout the whole thalamus.

(Supported by NSF research grant BNS-8209081 and NIMH research grant 04849 (ITD) and NIMH predoctoral fellowship 08312 (GRP)).

270.6 INTRACELLULAR STAINING OF ELECTROPHYSIOLOGICALLY IDENTIFIED NEURONS AND FIBERS IN VENTROBASAL THALAMUS (VB) OF CAT. C.-T. Yen and E.G. Jones. James L. O'Leary Division of Experimental Neurology & Neurological Surgery, and McDonnell Center, Washington University School of Medicine, St. Louis, MO. 63110.

Horseradish Peroxidase (HRP) filled microelectrodes have been used to reveal fine morphological details of physiologically characterized VB neurons and medial lemniscal fibers (ML) in barbiturate anesthetized cats. Cuff electrodes were used to stimulate the contralateral median and sciatic nerves. Natural stimuli were applied to skin, hairs and deep tissues. The ipsilateral ML was stimulated electrically at its medullary decussation and the ipsilateral first somatosensory cortex was stimulated over the forelimb representation. High impedance microelectrodes were filled with 10% Tris-buffered HRP. A high voltage electrometer was used to record and to inject. Frozen or Vibratome sections were reacted with cobalt intensified Diaminobenzidine. Twelve VB neurons, 12 ML fibers and one neuron in the reticular thalamic nucleus (RTN) have been stained.

Ten VB neurons were confirmed as thalamocortical relay cells (TC neurons) with a range of modality properties. Soma diameters ranged from 20 to 50 microns and dendritic fields 200-400 microns. Larger TC neurons with thick axons have thick proximal dendrites ending abruptly in a tuft of finer branches but no spines or protrusions. Smaller TC neurons have thinner, less highly but evenly branched dendrites with thin spines. No TC axons gave off local collaterals inside VB; several had collaterals ending in the RTN. Latencies of antidromic spikes of TC neurons ranged from 0.4 to 1.4 msec. Two small VB neurons which were not characterized physiologically had 3 to 4 thin twisted processes with occasional varicosities and may represent interneurons. The RTN neuron had a somatosensory receptive field. It had long, thin, sinuous dendrites. The thin axon collateralized profusely with many enpassant endings in RTN, VB and adjacent nuclei.

ML fibers demonstrated a range of modality properties and latencies of 0.5 - 1.3 msec. Individual fibers had 1 - 4 termination fields in VB. Within each field, densely packed boutons of variable size and shape were found (33 to 1240 per field). Almost all boutons were found within 50 - 400 micron long, rod-shaped termination field. Isolated boutons were rare. We conclude provisionally that TC neurons of VB have no intranuclear collaterals, but have collaterals in the RTN. There seem to be two morphological types of TC neuron and an interneuron. ML fibers terminate in very restricted but often multiple patches. Somatosensory RTN neurons terminate diffusely in VB and other nuclei.

Supported by NIH Grant Number NS10526 and McDonnell Center.

270.7 THREE-DIMENSIONAL RECONSTRUCTION OF THE TOPOGRAPHY OF THALAMOCORTICAL PROJECTIONS IN THE RAT. J.K. Chapin, D.S. Schlusselberg, W.K. Smith, and D.J. Woodward. Dept. of Cell Biology, U. Tex. Hlth. Sci. Ctr., Dallas, TX 75235.

This study was conducted to define the topographic relationships between the thalamus and precisely defined areas within the frontal and parietal cortex of the rat. Discrete injections of horseradish peroxidase (HRP) were made into the following cortical zones: fronto-medial agranular (FMAG), fronto-lateral agranular (primary motor (MI) cortex), granular zones (GZ's; containing the hindpaw, forepaw, and face areas of primary somatosensory (SI) cortex), dysgranular zones (DZ's; containing the limb and trunk areas of SI cortex), and SII cortex. Locations of retrogradely labelled neurons and thalamic nuclear boundaries, observed in serial coronal sections through the thalamus, were digitized with a graphics tablet. A computer was used to reconstruct this data into three-dimensional views of the thalamocortical organization.

Corticotopic patterns of labelled neurons were observed in the ventro-lateral (VL), ventral postero-lateral (VPL) and -medial (VPM), ventro-medial (VM), and posterior (Po) thalamic nuclei (defined in "The Rat Brain", by Paxinos and Watson, Acad. Press, 1982). After HRP injections into the different SI cortical GZ's, labelling was observed mainly within the VPL (paw areas) or VPM (face areas) in rostro-caudally oriented comma -shaped arrays of cells. The fore- and hindpaw arrays, but not the face arrays, widened rostrally into the VL, and formed the dorso-lateral edge of both VL and VPL nuclei. Injections in the DZ (SI limb areas) produced labelling in the dorso-medial part of rostral VL. Caudally, this labelling divided into a lateral branch in the dorsal (limb) area of the VPL and a medial branch in the lateral Po, just medial to the dorsal aspect of VPM.

Injections in MI cortex resulted in heavy labelling predominantly in the ventral region of the rostral VL. Caudally, this crescent-shaped continuous array of labelled cells divided into a ventral branch extending into VM, and a dorsal branch extending into the same general region in the lateral Po as was seen after DZ cortical injections. Specifically, neurons projecting to the SI forelimb cortex appeared to overlap with those projecting to the MI forelimb cortex. Injections into FMAG cortex produced similar crescent shaped patterns as MI injections, but more ventro-medial in VL and VM, and more medial in Po. Labelling from SII cortex was corticotopic but diffuse throughout VP and Po.

To summarize, these reconstructions indicated that thalamocortical projection neurons exist in rostro-caudally oriented arrays that may pass continuously through different cytoarchitecturally defined thalamic nuclei. Supported by grants NIAAA 3901, NS18041, and the Biol. Humanics Found.

270.8 THALAMIC CONNECTIVITY OF THE SOMATOSENSORY CORTICAL FIELDS OF THE LATERAL SULCUS OF THE MONKEY. D.P. Friedman, E.A. Murray and J.B. O'Neill*. Laboratory of Neuropsychology, NIMH, Bethesda, Md. 20205.

The thalamic connections of the cortical fields of the somatosensory system of macaques were investigated by making injections of tritiated amino acids or horseradish peroxidase into those fields. Injection sites in individual fields were initially selected by recording multi- and single-unit responses to mechanical stimulation of the body surface, or in a few cases, by direct visualization. The location of each injection was later verified on the basis of cytoarchitectural and connectional criteria. Additional monkeys with thalamic injections or cortical lesions supplied supplementary data.

There were three major findings: (1) The second somatosensory area (SII) receives its major thalamic input from the ventro-posterior inferior thalamic nucleus (VPI), with additional inputs arising from the parvocellular portion of the medial dorsal nucleus and from the central lateral nucleus. Previously reported inputs to SII from the caudal division of the ventroposterior lateral nucleus (VPLc) could not be confirmed. If they exist, they may arise only from the most ventral and caudal portions of VPLc that are adjacent to VPI, and from the ventroposterior medial nucleus (VPM). The finding in the monkey that SI and SII receive different thalamic inputs is consistent with the hypothesis that they process information in a sequential, rather than parallel manner, a notion that was based on previous reports that both fields received their inputs from VPLc. (2) Area 7b and the granular (Ig) and dysgranular (Id) insular fields, all now implicated in somesthesis, receive projections from the medial pulvinar (Pulm). Thus, the cortical territory that receives inputs from Pulm is much larger than previously believed and includes the somatosensory system. (3) Id receives major thalamic input from a continuous band of neurons that runs caudolaterally from the basal ventromedial nucleus (VMb) through VPI and the posterior nucleus (Po) to the anterior portion of the medial geniculate body. Additional inputs to Id arise from nucleus reuniens and the intralaminar nuclei.

We have confirmed the projections to area 7b from the oral nucleus of the pulvinar, to Ig from the suprageniculate and limitans nuclei, and to the retroinsular area from Po. Reciprocal thalamocortical and corticothalamic labeling was seen in each case where combined labeling was available.

Taken together, these results suggest that there is a complex array of thalamic inputs to individual cortical somatosensory fields outside of SI and that individual thalamic nuclei may project to a number of cortical fields within the somatosensory system.

270.9 CYTOARCHITECTONIC ORGANIZATION OF THE BRAINSTEM TRIGEMINAL NUCLEI IN THE MOUSE: CORRELATION WITH VIBRISSAL INPUTS AND POSTNATAL DEVELOPMENT. P.K.M. Ma* & T.A. Woolsey. Dept. of Anatomy & Neurobiology and McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110.

Representations of the facial vibrissae in the brainstem trigeminal nuclei of the mouse have been identified using histochemical staining methods. We have found that they can also be seen using routine Nissl staining methods. In Nissl stained celloidin sections of brainstems of both adult and neonatal mice, cytoarchitectonic organization was identified within subnuclei principalis, interpolaris, and caudalis in all three cardinal planes. The overall cytoarchitectonic patterns are identical to those seen using histochemical staining for succinate dehydrogenase. No distinct cytoarchitectonic pattern can be seen in subnucleus oralis. These patterns can first be observed by at least the third postnatal day in all three subnuclei. By the fifth or sixth postnatal day, a relatively mature pattern is present. Postnatal damage to vibrissal follicles by electrocautery altered these cytoarchitectonic patterns appropriately in all three subnuclei. Electrocautery of all the vibrissae at birth completely prevents the formation of the cytoarchitectonic pattern in all subnuclei. These results demonstrate that cytoarchitectonic representations of the facial vibrissae exist in all stations of the sensory pathway from the brainstem to the cortex. Identification of the cytoarchitectonic organizations in the brainstem trigeminal nuclei opens up new possibilities for studying development, function and plasticity of the trigeminal system using techniques with which histochemical staining methods are not compatible. (Supported by NIH Grant NS-17763 and the McKnight Foundation.)

270.10 INCREASED ^{14}C 2-DEOXYGLUCOSE UPTAKE IN CEREBELLUM, BRAINSTEM, AND THALAMUS DURING SENSORY STIMULATION OF RAT VIBRISSAE. F.R. Sharp, M. Gonzalez* and K.L. McKeown. Dept. of Neurosciences, UCSD School of Medicine, La Jolla, CA 92093

We have recently described the regions of rat brain which increase ^{14}C 2-deoxyglucose (2DG) uptake during stimulation of the "vibrissae" region of rat motor cortex (Sharp et al., JCN 208:255, 1982). In that study it was not possible to state if sensory feedback played a significant role in the 2DG changes observed. The present work was aimed at describing the changes of 2DG uptake in rat brain during mechanical sensory stimulation of the vibrissae.

Adult female Sprague-Dawley rats were briefly anesthetized and restrained to an animal board with tape. Animals were allowed to recover from anesthesia at least 12 hours. ^{14}C 2-deoxyglucose (20uCi/100 gm) was injected intravenously and stimulation of the right vibrissae begun. Stimulation was carried out with a mechanical, hand-held stimulator at 2-4 times per second in an anteroposterior direction to all of the vibrissae for 45 minutes. Animals were sacrificed and their brains frozen, sectioned, and autoradiographed as described previously.

The largest increases of ^{14}C 2DG uptake occurred ipsilateral to the stimulation in the ventral portions of the nucleus of the spinal tract of the trigeminal nerve including pars oralis (NTS_{VO}), pars interpositus, and pars caudalis. The ventral portion of the principal sensory trigeminal nucleus was also activated. Several regions of the cerebellar granule cell layer were activated ipsilateral to stimulation including large parts of crus II, and smaller parts of crus I, paramedian lobule, lobulus simplex, and anterior lobe hemisphere. 2DG uptake increased in deep layers of the superior colliculus contralateral to the side of stimulation. Other contralaterally activated structures include the posterior (PO) nucleus of thalamus, medial ventrobasal nucleus of thalamus (VB), and perhaps lateral parts of the medial posterior (POm) nucleus of thalamus. A broad increase in 2DG uptake also occurred in primary somatosensory cortex (SI) particularly layers IV and Vb.

Our data show that sensory stimulation of the vibrissae activates different structures than does stimulation of the vibrissae region of motor cortex. The only regions which appear to be activated by both include NTS_{VO}, ?POm, SI, and portions of the cerebellar hemisphere.

270.11 THE REPRODUCIBILITY OF 2DG PATTERNS IN MONKEY SI AND THEIR RELATIONSHIP TO SINGLE UNIT MAPPING DATA. S.L. Juliano, O. Favorov*, B.L. Whitsel, Dept. of Physiol., Univ. of N. Carolina, Chapel Hill, NC 27514.

This series of experiments sought to determine to what extent the complex, strip-like pattern of metabolic activity evoked by a controlled stimulus was reproducible from animal to animal. We also sought to define the relationship between the distribution of metabolic labeling and that of SI neurons possessing distinguishable functional properties. Extracellular microelectrode mapping penetrations were carried out in SI on the same animals which were used in metabolic mapping experiments. Four monkeys (macaca fascicularis) received an intermittent vertical displacement stimulus (15 hz) on the volar tip of digit 2. Two animals underwent the metabolic mapping procedure alone; 2 others were studied neurophysiologically and then underwent the 2DG procedure. Two additional animals underwent both microelectrode and 2DG mapping, but the 2DG experiment differed in that constant velocity (20 cm/sec) brush strokes were delivered to the volar radial hand. Reconstructions of the SI labeling patterns for the 4 animals stimulated with the flutter stimulus reveal remarkable similarities. Moreover, individual autoradiographs from comparable levels of SI for the different animals subjected to the same stimulus are nearly identical. The relationship of metabolic activity to single neuron properties is not simple. Metabolic label only occurred at cortical locations where the neurons possessed functional properties consistent with their activation by the stimulus used during the 2DG experiment. However, the single neuron observations obtained at loci between patches of 2DG label indicated that the properties of neurons in these sites were, in a number of instances, quite similar to those in the neighboring regions containing metabolic label. These findings suggest that the highly reproducible pattern of metabolic activity produced in SI by somatic stimulation is determined by multiple factors. Since labeling only occurs in regions where the single neurons possess RFs and submodality properties matching the stimulus, these properties must be a major determinant of the pattern. RF properties are clearly not the sole factor, however, since many neurons in the regions separating the strips possess properties consistent with activation by the 2DG mapping stimulus. We are currently investigating the possibility that an intracortical inhibitory mechanism together with the static properties of single SI neurons determine the spatial pattern of activity which uniquely represents a given somatic stimulus. (Supported by NS 10865 & ONR N0014-83K-0387)

270.12 DISCHARGE PATTERNS OF SOMATOSENSORY AND PERIARCUATE NEURONS IN AN ALTERNATING ATTENTION PARADIGM. G.Gücer, L. Viernstein,*R. Szymanski.* Dept. of Neurological Surgery, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Neurons of the somatosensory cortex S1 decrease their responsiveness to vibratory stimuli during sleep, particularly during REM sleep. To determine if such an inhibitory mechanism as involved in sleep can also reduce the responsiveness of post central neurons while the monkeys are attending to sensory input other than the vibratory system; we have recorded from 122 pre- and postcentral neurons while monkeys were engaged in an alternating attention paradigm. Monkeys were trained to discriminate between different frequencies of an auditory and also a vibratory stimulus. They were rewarded for pressing a lever for the contingent frequency and not the other. The discriminative nature of the task assured that they had to attend to each stimulus before making a decision. These auditory and vibratory games were alternated as cued by different colored lights.

The discharge pattern of 59 neurons located in Brodman's area 1, 2 and 3 did not reveal any significant change in the entrainment nor the strength of entrainment as monkeys shifted their attention back and forth between the vibratory and auditory stimuli. In two of our trained monkeys we extirpated the S1 cortical region and S1 plus areas 5 and 7 by subpial suction after single cell recording failed to show any significant changes in entrainment or responsiveness as animals shifted their attention between modalities. The day after surgery the monkeys played an alternating game and were capable of discriminating between 10 HZ and 100 HZ vibration as well as they did preoperatively. Our preliminary finding in 63 neurons in areas 4 and 6 showed two basic types of neural discharge. First, neurons that discharge rhythmically related to the rhythmic movement of the hand pressing the key. This neural response was independent of which modality was receiving the attention.

Secondly, we found neurons which started to discharge 200 to 300 MSECS after the onset of either the contingent auditory or contingent vibratory stimulus but not for both. Neurons of this type did not discharge rhythmically with the key hits but rather in a uniform manner but uniquely for the contingent auditory or vibratory stimulus. One other effect was seen as the attention was shifted, namely, the mean impulse rate of latter type neuron responding to vibration was compared with the neuron impulse rate while responding to tone for repeatable trials. For those neurons that enable for vibration a significantly lower impulse rate was found in the vibratory game than in the tone game. Neurons that enabled for tone had a significantly lower impulse rate in the tone game than in the vibratory game.

270.13 TACTILE DIRECTION DISCRIMINATION IN PRIMATES, G.K. Essick* and B.L. Whitsel. Dept. of Physiology, School of Medicine, Univ. of North Carolina at Chapel Hill, 27514.

The capacity of single S-I neurons of macaque monkeys and the capacity of human subjects to distinguish opposing directions of movement across the skin was studied employing experimental paradigms and data analyses based on sensory decision theory. A major advantage of this approach is that one obtains comparable behavioral and neurophysiological indices of directional sensitivity (i.e., they have the same metric).

A relative operating characteristic (ROC) curve, providing estimates of directional sensitivity, was generated for each of 18 neurons by using the responses of each neuron to multiple replications of the two opposing directions of movement. The rate-related neural response dimension which yielded the most directional information was determined. It was shown that even when the neural data sample is too small for ROC curve construction, reliable estimates of sensitivity can be computed since the distributions of neural responses are available to the experimenter.

ROC curves were generated for each of 5 human subjects who participated in a cutaneous direction discrimination task. It was shown that the SDT "Gaussian equal-variance" hypothesis was applicable to the data. Furthermore, experimental designs which account for among-subject as well as among-session differences in perceptual sensitivity possess power sufficient to allow quantitation of the influences of variations in a single stimulus parameter on tactile directional sensitivity.

Data from 90 S-I neurons and 9 human subjects were consistent with the following conclusions. Cutaneous directional sensitivity of human subjects and of one class of S-I neurons, the "direction invariant" neurons, (i) is maximal when the stimuli move between 5.0 and 30.0 cm/sec, and (ii) increases as the length of skin traversed is increased. Furthermore, human cutaneous directional sensitivity is greater on body regions receiving a dense cutaneous innervation and is independent of the orientation of the moving tactile stimulus. Members of a second class of directionally selective S-I neurons, the "direction variant" neurons, signal movement toward or away from a given region within the receptive field, and thus do not unambiguously encode stimulus direction on the skin.

Of the two classes of directionally sensitive S-I neurons studied, only the direction invariant neuron class appears to possess properties consistent with human tactile directional sensitivity and its dependency on stimulus parameters.

(Supported, in part, by NS10865, DE02668 and Dept. of Navy Contract N0014-83-K-0387.)

AGING I

271.1 INTRACELLULAR STUDIES OF THE AGE-RELATED DEFICIT IN HIPPOCAMPAL FREQUENCY POTENTIATION: APPARENT CALCIUM SATURATION IN SYNAPSES OF AGED RATS. P.W. Landfield, T.A. Pitler*, M.D. Applegate* and J.H. Robinson*. Dept. of Physiol. & Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103.

In another paper at this meeting (Pitler & Landfield) we report that hippocampal frequency potentiation appears to depend upon residual Ca^{2+} accumulation in presynaptic terminals. In this report we describe quantitative analyses of the timecourse of synaptic and membrane events associated with frequency potentiation in hippocampal slices from aged and young rats.

The data were obtained in intracellular recordings from approximately 60 neurons in the CA1 pyramidal cell layer (stratum pyramidale) from 15 aged rats (27-30 mo-old) and 20 young-mature rats (5-8 mo-old). Barrier-reared, male Fischer rats from the NIA colony at Charles River were used. Aging animals exhibiting overt pathology were not included in these studies. Cells were excluded from analysis if they failed to meet our criteria for healthy cells, or for stable penetrations (e.g., less than 15 Mohm input resistance, less than 50 mV stable resting potential, less than 60 mV spike, cells held for less than 15 min).

In each cell, EPSP amplitude, input resistance and membrane potential were measured before, during and following a 4 min period of 10 Hz stimulation of the Schaffer collaterals. Measures were obtained at 5, 15, 30, 45 and 60 sec, and at 2, 3 and 4 min of 10 Hz stimulation.

A highly significant reduction was found in the amount of EPSP potentiation in cells from aged rats, both in terms of maximal potentiation and in the duration of potentiation. As noted in a separate report in this meeting, potentiation of EPSP amplitude during stimulation is closely correlated over time with increased membrane conductance changes. This conductance change is apparently due to a Ca^{2+}-activated K^+ conductance, and therefore argues that EPSP potentiation is also dependent on intracellular Ca^{2+} concentration.

In cells from aged rats, this correlation was maintained, and both EPSP potentiation and membrane K^+ conductance changes were "blunted" during stimulation. These findings suggest that Ca^{2+}-activated processes in hippocampal neurons are less responsive in aged animals. We previously found that elevating Mg^{2+} in the bathing medium, which is believed to reduce Ca^{2+}-binding at active intracellular sites, strongly increased frequency potentiation in slices from aged rats (Landfield, Soc. Neurosci. Abstr., 1981). Taken together, these data are consistent with the hypothesis that hippocampal synaptic frequency potentiation is blunted in aging because of Ca^{2+}-saturation of available Ca^{2+} sites in synaptic terminals. Such saturation could result from an elevation of intracellular $[Ca^{2+}]$. (Supported by AG 01737)

271.2 DIFFERENCES BETWEEN AGED AND YOUNG MICE IN REACTION TO CAFFEINE AND IN ADENOSINE RECEPTORS. L.D. Middaugh, N.S. Buckholtz and D.K. Ingram.* Medical University of South Carolina, Dept. of Psychiatry and Behavioral Science, Charleston, SC 29425 and Geront. Res. Center, Baltimore, MD 21224.

Several reports indicate that the elderly have atypical responses to numerous drugs. Although the stimulatory effect of caffeine on the elderly presumably differs from that on younger individuals, a systematic comparison is unavailable. Recent evidence suggests that caffeine-induced changes in locomotor activity of rodents is mediated by its antagonist action on adenosine receptors. In the present study, we examined the effect of caffeine on locomotor activity of young (10 to 12 mos.) and aged (28 to 36 mos.) C57BL/6J mice and also compared the two age groups on the binding of N^6-cyclohexy [^{3}H] adenosine ([^{3}H]CHA) in four brain regions.

Locomotor activity was assessed for three hours following intraperitoneal injections of caffeine (5, 15, 20, or 45 mg/Kg) into mice previously habituated to the activity monitoring apparatus for one hour. Activity of both age groups was elevated by the 15 mg and 30 mg/Kg doses; however, the extent of elevation above pre-drug levels was greater for aged than for young mice. This was particularly evident at later time periods when ratios of post- to pre-injection activity counts ranged from 1.5-2.0 for aged mice compared with 0.8-1.0 for young mice. In spite of the greater degree of activity elevation for the aged group, the time course for elevated activity was similar for both groups.

Adenosine receptor binding was determined in cortical, hippocampal, striatal, and cerebellar tissue from mice in the above age groups using [^{3}H] CHA as the ligand. For both age groups, the greatest amount of binding occurred in hippocampus and cerebellum. Binding did not differ according to age in the cortex, hippocampus, or striatum; however, it was 15% higher in cerebelli of aged than young mice

Although young and aged mice appear to differ in their reaction to caffeine and in [^{3}H] CHA binding, whether or not the two age differences are functionally related remains to be determined. (Supported in part by P.H.S. Grant MH37747 to N.S.B.)

271.3 VOLUME OF THE DENTATE GYRUS MOSSY FIBER PROJECTION SYSTEM IN REGIO INFERIOR OF AGING F344 RAT. V.B. Moyer*, P.D. Coleman, M.J. West, and S.J. Buell* (SPON: T. Pasternak). Depts. of Anatomy and Neurology, University of Rochester Medical Center, Rochester, New York 14642 and Institute of Anatomy B, Aarhus Univ., Aarhus, Denmark.

The volume of the mossy fiber projection from dentate gyrus to regio inferior of the hippocampus was measured in seventeen male F344 rats at ages from 4 to 33 months. The mossy fiber system was visualized with the Timm stain. Volumes were determined stereologically from coded horizontal sections by the point count method. The hilus region was excluded. The average total volume determined for all animals was 2.36 mm^3 throughout the entire dorso-ventral extent of the mossy fiber system. The average values for each age in mm^3 were 2.22, 2.53, 2.20, 2.18 and 2.52 for ages 4, 12, 20, 27 and 33 months, respectively. Measures of the infra-, intra- and supra-pyramidal subdivisions of the mossy fiber system gave mean volumes of 0.34, 0.21 and 1.81 mm^3, respectively. Volumes of these subdivisions in individual animals varied in parallel with the volumes of the total mossy fiber system.

Supported by the National Institute on Aging (AG1121 and AG2680) and by The Institute of Anatomy, Aarhus University

271.4 VOLUMES OF THE SUBDIVISIONS OF THE DENTATE GYRUS AND HIPPOCAMPAL REGION IN AGING F344 RAT. M.J. West and P.D. Coleman. Institute of Anatomy B, Aarhus Univ., Aarhus, Denmark and Department of Anatomy, University of Rochester Medical Center, Rochester, NY 14642.

Much of the recent data on cells, synapses and other structures in the dentate gyrus and hippocampus as a function of age are density or volume fraction data. In order to estimate total numbers, volumes or surface areas of cells, synapses, vessels, etc. as a function of age the total volumes of the subregions of the dentate gyrus and hippocampus must be known. These volumes have been determined in four 12 and four 33 month old male F344 rats (data for intermediate ages will be available at the presentation of this paper). The subdivisions were visualized with the Timm stain. Boundaries of the following regions were manually traced from 35-40 coded horizontal sections for each animal through the dorso-ventral extent of the dentate gyrus and hippocampus: 1) hilus, 2) layer of granule cells, 3) commissural-associational zone of dentate gyrus molecular layer, 4) perforant path zone of molecular layer and 5) hippocampus plus subiculum (all layers). The resulting drawings were automatically traced by an image dissector and PDP-11 computer system. The computer derived three dimensional reconstructions and volumetric quantifications on the basis of these data. Volumes in mm^3 were: hilus, 12 mo. (Y)-2.65, 33 mo. (O)-2.73; layer of granule cells, Y-2.41, O-2.61; commissural-associational, Y-2.42, O-2.47; perforant path, Y-7.30, O-7.39; hippocampus & subiculum, Y-40.64, O-43.27. Volumes were always greater in the 33 month animals than in the 12 month animals with old/young x100 ranging from 101% (perforant path zone) to 108% (layer of granule cells). Analysis of variance showed a significant region main effect, a non-significant age main effect and a significant age x region interaction. These data suggest that some of the regions analyzed beome significantly larger with age while others do not. In those regions which become significantly larger with age the interpretation of density or volume fraction data may require modification. Data from intermediate ages will clarify whether changes in volume as a function of age are linear.

Supported by the National Institute on Aging (AG-1121) and by The Institute of Anatomy B, Aarhus Uniyersity.

271.5 REGENERATION OF CENTRAL CATECHOLAMINE FIBERS IN AGED RAT BRAIN. Carol Phelps and John R. Sladek, Jr. Dept. of Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, N.Y. 14642.

Aging is often accompanied by degenerative changes in the CNS which can include a loss of neurons, synapses and/or transmitter content. However some evidence suggests that central catecholaminergic (CA) neurons may retain a degree of plasticity in response to alterations in their target neurons. This response is manifested by an apparent hyperinnervation of peptidergic targets in the hypothalamus (Sladek et al., Peptides 1:141, 1980). In addition, an earlier study demonstrated regenerative capacity in a CA pathway in young rats (Turpen and Sladek, Cell. Tiss. Res. 187:449, 1978). Taken together, these observations suggested that regenerative and/or sprouting activity may occur in aged brain. To test this hypothesis, CA histofluorescence patterns in the area of a neurosurgical lesion were evaluated.

Transection of the ascending medial forebrain bundle (mfb) was accomplished using rotation of a handhewn knife, positioned vertically in the brain according to three-dimensional stereotaxic coordinates (Paxinos and Watson, 1982), achieving partial loss of the CA innervation of the supraoptic (SON) and paraventricular (PVN) nuclei. The area damaged by surgical intervention was examined in 3, 20, and 30 month-old male Fischer 344 rats, at matched intervals after lesion placement. Brains were prepared for fluorescence histochemistry by formaldehyde/glutaraldehyde (FAGLU) and aluminum/formaldehyde (ALFA) perfusion fixation.

Two simultaneous processes were evident in the lesion site: CA fibre degeneration and vigorous regrowth of axons. By 4 days following surgery, fine varicose fiber growth was observed, passing into and through the scarred region. Further regenerative activity was progressive, in rats of all ages, between 4 and 14 days after lesion placement. Although the lesion site showed greater necrosis and inflammatory reactivity in aged animals, regrowth was demonstrable, by these sensitive histofluorescence methods, in the mfb-lesioned areas of aged brains. In fortuitous sections, new fibers could be clearly identified as growth from damaged axons.

Progressive growth, and fiber invasion of a surgically scarred area, imply robust plasticity in CA pathways, which persists in aged brain. Possible reinnervation of target sites, at longer postsurgical intervals, is currently being evlauated.

Supported by USPHS Grant AG 00847 (JRS).

271.6 EFFECT OF DEVELOPMENT AND AGING OF THE RAT ON UNIDIRECTIONAL PALMITATE FLUX INTO BRAIN. H. Tabata, A.S. Kimes, J.M. Bell, and S.I. Rapoport. Lab of Neurosciences, Gerontology Res. Ctr., Natl Inst on Ag., NIH, Baltimore City Hospitals, Balto., MD 21224.

Palmitate flux into a stable 4 hr-brain compartment is proportional to the regional cerebral metabolic rate for glucose (rCMRglc) (Kimes et al., Brain Res. in press) and can be reduced by barbiturate anesthesia (Kimes and Rapoport, Neurosci. Abst. 8: 1003, 1982). Because palmitate flux is proportional to rCMRglu, it was of interest to measure flux in awake Fischer-344 rats at the ages of 1,3,12, 24 and 34 mo. In addition, because rCMRglc and regional cerebral blood flow (rCBF) show different patterns for aging animals, it was of interest to determine if palmitate flux varied with development and aging like either rCBF or rCMRglc.

Femoral arterial and venous catheters were implanted under ether anesthesia. Rats were allowed 4 hr to recover from anesthesia, following which 450 µCi/kg [14C]palmitate was injected i.v., and timed arterial blood samples were collected. At 4 hr after injection, rats were killed and brains were frozen. Brain radioactivity was determined in 43 regions by quantitative autoradiography. Plasma radioactivity due to [14C]palmitate was determined by extraction and thin layer chromatography. Unesterified plasma palmitate was determined by gas chromatography. Unidirectional flux of plasma palmitate into the brain (J, µmol/g.sec) was calculated from the equation: J=kCplasma, where k=transfer constant for plasma palmitate to brain, and Cplasma= plasma concentration of palmitate. k was determined by the equation $k=C^*brain/\int_0^t C^*plasma\,dt$, where C*brain = the brain radioactivity at 4 hr after injection and $\int_0^t C^*plasma\,dt$ = integral plasma [14C]palmitate between 0 and 4 h.

J declined 15 to 30% between 1 and 3 months in all regions, rose 15 to 35% between 3 and 12 months and then declined 20 to 30% by 24 months. Changes in regional J between 3 and 12, 12 and 24, and 24 and 34 months were similar to changes in rCBF as reported by Ohata et al. (Brain 104:319-332, 1981) although rCBF increased between 1 and 3 mo. Unlike both rCBF and rCMRglc, regional palmitate flux at 1 mo was higher than that at 3 mo. Considering the fact that the maximum rate of myelin deposition for rats occurs at 20 days of age (W.T. Norton and S.E. Poduslo, J. Neurochem. 21: 759-773,1973), a higher flux at 1 mo than at 3 mo is related possibly to myelinization. This is supported by a significantly larger flux into white matter compared to gray in 1 mo versus the older animals ($p < .05$). The ratios of palmitate flux into white matter as compared to gray matter was 0.61 at 1 mo, 0.49 at 3 mo, 0.51 at 12 mo, 0.46 at 24 mo, and 0.50 at 34 mo.

271.7 NEUROMUSCULAR JUNCTION REMODELING IN THE RAT SOLEUS DURING AGING: A MORPHOMETRIC STUDY. C. A. Cardasis* and D. M. LaFontaine* (SPON: E. Taber-Pierce) Dept. of Anatomy, Univ. of Mass. Medical School, Worcester, MA 01605.

Ultrastructural remodeling of neuromuscular junctions (NMJ), with evidence of focal denervation and reinnervation occurs in normal rat soleus during aging (Cardasis & Padykula, '81; Cardasis, Anat. Rec., in press). From 3 to 9 months (Group I) this may relate to body growth and increased workload. When growth slows (11-19 months, Group II) the balance between de- and reinnervation changes: NMJs with exposed junctional folds (focal denervation) increase from 61% to 100%. We sought to quantitate junctional fold exposure in individual NMJs, to characterize spacing of normally innervated versus exposed folds using TEM, and to compare soleus with diaphragm, which undergoes less intense age-related reorganization, by study of ChE stained whole mounts. Morphometric analysis of longitudinally oriented EM composites of 20 Group I and 21 Group II NMJs showed, in Group II, significant increase in fold exposure in individual NMJs (Group I = 20%, Group II = 69%, $P<.0001$.), and a trend toward clustering of normal folds and separation of exposed folds (Distance (μm) between normal folds: I = 0.562±0.12, II = 0.451±0.10. P=.038. Exposed folds: I = 0.554±0.12, II = 0.685±0.16. P=.024). This data suggests increased area of postsynaptic membrane in the normal folds of Group II. The area of soleus and diaphragm endplates was determined in ChE preparations in 3 rats each at 1, 3, 7, 11, and 15 months, measuring 20 NMJs/muscle/rat/age:

Soleus:	246±62	677±214	728±102	1399±495	930±142 μm
Diaphragm:	198±22	408±34	763±28	799±58	842±131 μm

With age, there was increase of endplate area, myofiber size, and complexity of branching of primary folds. In the soleus by 11 months degeneration was evident, as suggested by diffuse rather than discrete localization of ChE reaction product, or sometimes both within an individual NMJ, and wide separation (approximately 3 μm) of multiple NMJ regions on individual muscle fibers. Diaphragm NMJs did not become as complex nor degenerate as rapidly as soleus. The net effect of remodeling of soleus NMJs appeared to be increase of normal synaptic area during the first year and decrease of normal synaptic area during the second year.
Supported by NSF Grant #BNS-8207829.

271.8 SPONTANEOUS NEURONAL ACTIVITY IN THE NEOCORTEX AND STRIATUM OF YOUNG AND OLD RATS. W.C. Stern, W. Pugh*, and P.J. Morgane. Burroughs Wellcome, Research Triangle Park, NC 27709 and Worcester Fndn. Exptl. Biol., Shrewsbury, MA 01545.

The present study compared the spontaneous activity of single units in the frontal cortex and the striatum of 20 young (5-11 mo.) and 10 old (25-31 mo.) Fisher 344 albino male rats. These structures were selected because of their importance in cognitive and motor events, measures which often change during aging, and because they represent regions of low (cortex) and high (caudate) spontaneous discharge rates.

Rats were anesthetized with 1000 mg/kg urethane i.p., and single neurons were recorded extracellularly using glass micropipettes. The major findings obtained from computer analyses of spike activity are summarized in the table below:

	Frontal Cortex		Striatum	
	Young	Old	Young	Old
# Cells	105	121	42	28
Spikes/Sec	4.1±.4	3.3±.3	18.2±1.8	12.3±1.9*
ISI (msec)	634±87	1088±190*	218±74	728±393
Variability Ratio	1.26±.04	1.28±.05	.66±.06	1.16±.13***
% Bursts	9±1	7±1	10±2	17±3
% Pauses	13±1	11±1	6±1	8±1

*p < .05, **p < .01, ***p < .001 (2-tailed t-tests): Above values are mean ± S.E.; ISI = inter-spike intervals, variability ratio = mean/SD of the ISI

These results show a 20-35% decrease in average discharge rate and a consequent lengthening of the mean ISI in the cortex and the striatum of old rats. Also, the variability of discharge rates was markedly increased in the striatum of the old rats. In young rats, most striatal cells exhibit a sustained high rate of discharge, often 15-25/sec. However, these rates were not sustained in many of the striatal cells from the older rats.

The present results from cortex and striatum are consistent with two prior studies which showed a decrease in the average discharge rate for locus coeruleus neurons (Olpe, Brain Res. 1982) and for Purkinje cells of the cerebellum (Rogers et al., Neurobiol. Aging, 1980). This suggests that in the aged rat, decreased rate of neuronal discharges are likely to be characteristic of many brain regions. Moreover, these changes may be associated with a decreased metabolic rate observed in the aged nervous system. (Supported by NIA contract N01-AG-0-219 and funds from Burroughs Wellcome Co.).

271.9 CONDUCTION VELOCITY OF CORTICALLY-PROJECTING NUCLEUS BASALIS NEURONS DECREASES WITH AGE. J. Rogers,[2] G. Aston-Jones,[1] T. Dinan,[1]* R. Shaver,[1]* and D.E. Moss.[3] [1]Center for Neurobehavioral Sciences, SUNY, Binghamton, NY 13901, [2]Dept. of Neurology, U. Mass. Medical School, Worcester, MA 01605, [3]Dept. Psychology, U. Texas, El Paso, TX 79968.

Cholinergic neurons of the basal forebrain (nucleus basalis of Meynert in primates, nucleus basalis magnocellularis in rodents)(nBM) have come under recent experimental scrutiny because of their possible involvement in the etiology of Alzheimer's Disease. A parallel study (Aston-Jones et al., this volume) has examined normative conduction characteristics of cortically-projecting nBM fibers. In the present research, we extend those data to changes with age. Single nBM units were antidromically driven from a frontal cortex stimulating electrode in chloral hydrate anesthetized rats, 3 mos, 18 mos, or 28 mos old. The collision technique was used to verify antidromicity of driven spikes and to insure that segments of the same axon were being tested at different cortical depth placements of the stimulating electrode. By sampling conduction latencies from superficial to deep cortex in 300 u increments, it was possible to derive estimates not only for overall conduction velocity of cortically-projecting nBM fibers, but also for conduction velocity within gray matter (i.e., superficial to deep cortex) and white matter (i.e., deep cortex to nBM). All antidromically driven cells were histologically localized to the globus pallidus area, corresponding to anatomic maps of acetylcholinesterase-positive cells. Sixty-five such neurons are included in the present report.

Overall, conduction latencies for young rats were significantly shorter ($P < .001$) than for middle-aged and old rats. Further analysis at the various stimulating electrode depths, however, revealed that the conduction velocity in gray matter was virtually identical for all age groups. The difference in overall latencies was therefore due almost entirely to a two- to four-fold decrease with age in subcortical conduction velocity.

Since the estimated white matter conduction velocity for young rats (2.3 m/sec) is consistent with fine, myelinated fibers, one possible explanation for our data is that aging occasions a partial demyelination of cortically-projecting nBM axons. Further research, particularly on the ultrastructure of nBM axons, will be necessary to determine the cellular bases for age-related decline of nBM cortical afferents.

Supported by NINCDS Grant NS19360 and BRSG Grant SO7RR7149-09 to G. A.-J., and BRSG Grant 6-32840 to J.R.

271.10 CHOLINE EFFECTS ON NEUROMYAL TRANSMISSION IN MATURE AND AGED CBF-1 MICE.
Hiroshi Hasuo, Alexander G. Karczmar and Tashihiko Nishimura, Dept. of Pharmacology, Loyola Univ. Med. Center, Maywood, Il.

This study concerns the effects of choline, one of acetylcholine precursors, on neuromyal transmission in mature (1 year old) and aged (2 year old) inbred CBF-1 mice (Hill, Charles River Digest, 20:1, 1981). Isolated phrenic nerve-diaghragm preparations were employed and microelectrodes were used to measure miniature endplate potential frequency (mEPPf) and quantal content (QC). We have previously shown that presynaptic function and effectiveness of neuromyal transmission are decreased in aged animals (Hasuo & Karczmar, Soc. Neurosci. Abstracts, 8:440, 1982; Fed. Proc. 42:750 1983). At this time we tested whether or not choline can improve the presynaptic function in vitro and whether the effect, if any, is age-related. Higher (0.5mM) concentrations of choline evoked immediate postsynaptic effects; in confirmation of earlier data (cf., eg., Adams, 1975), these effects included depolarization and/or curarimimetic action. At concentrations of 0.05 to 1mM, choline did not exert appreciable effects on QC or mEPPf whether in mature mice or old mice which exhibit depressed mEPPf and QC values (Hasuo and Karczmar, o.c.). However, at concentrations of 50 and 300μM, choline prevented or reversed decrease in QC and in mEPPf induced by prolonged (2 hrs.), repetitive (10Hz) presynaptic stimulation. In non-treated animals the decrease in QC induced by repetitive stimulation amounted to 34 and 21 percent of control values in mature and aged mice, respectively; similarly induced, decrease of mEPPf amounted to 30 and 25 percent of controls in mature and old mice, respectively. Depression of QC induced by repetitive stimulation was diminished in 50μM and reversed in 300 μM choline solution in mature animals; it was reversed in 300μM choline solution in both mature and old mice. Similarly, choline 300μM, prevented mEPPf decrease induced by repetitive stimulation. Repetitive stimulation did not have a significant effect on mEPPf amplitude in either age group, nor did choline superfusion affect this parameter. These preliminary data suggest that 1) decrease of QC and mEPPf values after stimulation was smaller in aged than in mature mice, and 2) antagonism of these effects by choline was not significantly different in the two age groups. As in resting state exogenous choline seems to be incorporated into phospholipids rather than ACh (Kindel et al., Fed. Proc. 42:1983), present data suggest that increased activity augments choline participation in presynaptic ACh pool.

Supported in part by BRSG Grant No. 104, and Potts Foundation Grant. CBF-1 mice were kindly supplied by NIH Institute on Aging

271.11 PHYSIOLOGICAL CHARACTERISTICS OF PLANTARIS MOTOR UNITS OF YOUNG AND OLD RATS. F. Pettigrew* and P.F. Gardiner, Neuromuscular Research Group, University of Montreal, Montréal, Qué., Canada, H3C 3J7

Several alterations in adult neuromuscular structure and function with aging have been previously reported, including losses in spinal motoneurones, physiological and structural changes at the neuromuscular junction, and biochemical and physiological changes in skeletal muscles. These changes would be expected to manifest themselves functionally at the level of the motor unit. In this study, the isometric contractile characteristics of plantaris (PL) single motor units (MU) were studied in 6 young (3 mos) and 4 old (33 mos) male Fisher rats. MU were functionally isolated by stimulation of single PL axons following dissection of ventral root filaments in the lumbar spinal cord (L4-L5) MU characteristics are presented in the Table below as means ± S.D., with range in parentheses. PL from old rats were slightly (16%) smaller in wet weight, while whole-muscle tetanic tension was 56% smaller ($p < 0.01$). The variability in tetanic tensions among fast MU was higher in old animals, due to the presence of a significant number of high-tension units (> 30 g) in the latter. Old fast MU showed CT and fatigue resistance characteristics comparable to those in young rats. Slow MU were more easily obtainable from old (10 of 22 MU) than young (2 of 29 MU) animals, and generated larger tetanic tensions in the former. As with the fast MU, no differences were apparent in slow MU, CT or fatigue characteristics between the age groups. The data are consistent with a scheme of neuromuscular remodelling occurring during aging which includes motoneurone death, subsequent sprouting of remaining motoneurones, and muscle fiber-type conversion.

Supported by grants from MRC Canada, and NSERC Canada.

		CT (ms)	Tetanic tension (g)	Tetanic tension (% muscle)	Fatigue index
Young	S (n=2)	25	1.0± 0.1 (0.9- 1.1)	0.2± 0.1 (0.1- 0.2)	50
	F (n=27)	14.8± 1.1 (13.0-16.0)	13.2± 9.2 (1.3-34.2)	2.2± 1.4 (0.2- 5.0)	67.5±10.9 (47.0-82.0)
Old	S (n=10)	27.1± 2.3 (25.0-30.0)	3.4± 1.1 (1.7- 5.0)	1.6± 0.9 (0.6- 3.4)	50
	F (n=12)	15.4± 1.6 (13.0-18.0)	17.3±17.4 (0.7-48.7)	5.6± 6.9 (0.5-22.6)	70.7±13.5 (53.0-94.0)

271.12 3,4-DIAMINOPYRIDINE PARTIALLY REVERSES THE AGE-RELATED DECLINE IN SYNAPTOSOMAL CALCIUM UPTAKE. C. Peterson and G.E. Gibson, Cornell Univ. Med. Coll. Burke Rehabilitation Center, White Plains, NY 10605

The molecular basis of the age-related cognitive changes is unknown. Since several lines of evidence suggest that senescence may alter calcium homeostasis, calcium-45 uptake was determined in brain synaptosomes from aged rats. Incubations were terminated by vacuum filtration after the addition of EGTA/ruthenium red. In low potassium (5 mM-KCl) media, aging decreased calcium uptake from 100.0% (6 months) to 76.7% (15 months) or 58.6% (27 months). In 31 mM-KCl media uptake declined when the age increased from 6 (100.0%) to 15 (67.8%) or 27 (55.8%) months. Since our previous studies demonstrated that 3,4-diaminopyridine stimulates synaptosomal calcium uptake, we tested its efficacy on these age-related deficits in calcium metabolism. 3,4-Diaminopyridine elevated the potassium-stimulated calcium uptake in high K^+ media 13.1%, 23.8% or 140.0% in synaptosomes from 6, 15, or 27 months old rats, respectively, when compared to non-drug treated synaptosomes of the same age. The calcium that is bound to the plasma membrane externally was measured with incubations that are terminated by ruthenium red and filtration. This binding increased with age from 100.0% (6 months) to 116.1% (15 months) or 141.4% (27 months). 3,4-Diaminopyridine reduced this superficial binding at 15 or 27 months to 109.3% or 121.4%, when compared to the 6 month old rats. Thus, age-related changes in calcium homeostasis that may alter neuronal metabolism can be ameliorated by treatment with 3,4-diaminopyridine.

Supported in part by grants NS16997, NS03346 and the Winifred Masterson Burke Relief Foundation.

271.13 RAPID INDUCTION OF CEROID FORMATION IN RAT HIPPOCAMPUS BY LEUPEPTIN. G.O. Ivy, F. Schottler, M. Baudry, and G.S. Lynch. Dept. of Psychobiology, Univ. of California, Irvine, Irvine, CA 92717.

The accumulation of pigmented lipid particles in the brain has long been known to coincide with aging, as well as with various metabolic disorders, such as neuronal ceroid lipofuscinosis. While the large lipid conglomerates are thought to arise in lysosomes, the series of events leading to their formation remains unclear. We now report that an inhibitor of thiol proteases, leupeptin, causes the rapid accumulation of lipofuscin-like material in both normal and denervated rat hippocampus.

Continuous infusion of leupeptin (20µg/hr) or saline (.05 µl/hr) into the lateral ventricle of 40-90 day old rats was accomplished with an indwelling cannula connected to an osmotic minipump. The rats were sacrificed from 3 to 14 days later and their brains prepared for electron microscopy using conventional procedures. Light microscopic examination of semi-thin sections stained with toluidine blue revealed the presence of numerous dense bodies in a perinuclear position in the several types of hippocampal neurons. Astroglial cells also contained accumulations of this material. Preliminary studies suggest that this effect does not occur in cerebellum. Electron microscopic analysis indicated that the perikarya and dendrites of hippocampal neurons from drug treated animals contained electron dense bodies that in size and appearance closely resembled lipofuscin. Denervation of the dentate gyrus by removal of the entorhinal cortex resulted in deposits of lipofuscin-like material throughout the dendritic trees, as well as in the granule cell bodies and in numerous glial cells in rats treated with leupeptin. None of these effects was obtained in rats infused with saline.

It should be noted that the cannula caused some damage to the hippocampal fimbria and thus the observed effects may reflect an interaction between leupeptin and denervation, even in the animals without entorhinal lesions. Experiments with cannulae implanted at various sites in brain combined with analysis of several brain regions are now in progress.

Since leupeptin blocks thiol proteases, known to be present in lysosomes, these results suggest that inhibition of lysosomal activity causes a rapid accumulation of ceroid material in the brain. It is possible that these findings have significant implications for the origins of the ceroid substances that accumulate during aging and disease.

(Supported by NIA grant AG00538.)

271.14 ELEVATED ^{3}H-IMIPRAMINE BINDING IN AGED HUMAN AND MOUSE BRAIN. J.A. Severson. Department of Psychiatry, University of Southern California, School of Medicine, Los Angeles, CA 90033.

The tritiated form of the antidepressant imipramine binds to a site on serotonergic nerve terminals that modulates serotonin reuptake. This site is assumed to be a locus of the clinical efficacy of imipramine. The brain binding site and a pharmacologically similar site on platelet membranes appear to be regulated similarly and, therefore, the platelet site has been suggested to reflect the state of brain imipramine sites. Although conflicting evidence exists concerning the brain site, the number of platelet imipramine sites is reduced in clinical depression.

The elderly, as a group, are more prone to depression and suicide than the general population. An analysis of imipramine binding sites in brain might determine if alterations in this depression-related biological marker reflect a predisposition in the elderly to depression.

Binding sites for ^{3}H-imipramine were determined by filtration assay using washed membrane preparations. In a sampling of 24 human brain specimens ranging from 17 to 100 yr., hypothalamic ($r=0.76$, $P<0.0001$), frontal cortical ($r=0.63$, $P<0.002$) and parietal cortical ($r=0.60$, $P<0.002$) imipramine Bmax was elevated with increasing age. The Kd of the site for ^{3}H-imipramine was unchanged with age. In all three regions, Bmax doubled between 17 and 100 yr. Analysis of covariance confirmed the age effect on Bmax and determined that other factors associated with human autopsy specimens, such as time from death to autopsy, length of storage as frozen tissue, cause of death and sex, did not significantly affect binding. Hypothalamic samples were also analyzed for the regulation of ^{3}H-imipramine sites by Na^+ and Cl^-. The ability of Na^+ to enhance ^{3}H-imipramine binding was impaired with age ($r=-0.43$, $P<0.04$), but Cl^- enhancement of binding was intact ($r=0.04$, n.s.).

Cerebral cortical ^{3}H-imipramine Bmax was elevated 45% in 26 mo. male C57BL/6J mice, relative to 3 and 10 mo. mice. The Kd for imipramine was similar in the three ages examined. This suggests that the aged mouse can serve as a model for changes in human ^{3}H-imipramine binding during aging. In general, the data suggest that elevations in imipramine sites and impaired regulation of the sites may contribute to alterations in central serotonin function and, in turn, may predispose the elderly to depression.

271.15 POSTSYNAPTIC SEROTONIN BINDING SITES IN POSTMORTEM HUMAN BRAIN THROUGHOUT THE LIFESPAN. J Marcusson*, DG Morgan*†, B Winblad* and CE Finch†. (SPON: SP Bessman). Dept of Pathology, Univ. of Umea, Umea S-90187, Sweden, and †Andrus Gerontology Center, Univ. of Southern California, Los Angeles, CA 90089-0191, USA.

Postmortem human material was analyzed for S-1, S-2, S-2A, and S-2B binding sites in frontal cortex and hippocampus, and 5-HT, and 5-HIAA levels in frontal cortex and hypothalamus.

S-1 binding was assessed with ^{3}H-serotonin using 1 mM nonradioactive serotonin to define nonspecific binding. The density of S-1 sites declined with age in both frontal cortex (n=20; r=-0.49; p<0.05) and hippocampus (n=14; r=-0.62; p<0.02). Linear regression analysis indicated a 30% decline between the ages of 20 and 100 years. The average B_{max} in both regions was 470 fmoles/mg protein and the K_d was 3.70 nM. No age related change in K_d was observed.

S-2 binding was determined with ^{3}H-spiperone. For the total S-2 site, 0.5 µM ketanserin was used to define nonspecific binding. To discriminate S-2A from S-2B binding, displacement curves with methysergide were performed on the same tissue (see Morgan et al, this meeting, for definition of S-2A and S-2B binding). The total S-2 binding declined by 41% (20-100Y) in frontal cortex (n=24; r=-0.73; p<0.01) but not in hippocampus. The average B_{max} for total S-2 binding was 360 fmoles/mg protein in frontal cortex, and 162 fmoles/mg protein in hippocampus. No correlation was found between the two regions. The K_d for ^{3}H-spiperone was approximately 1 nM in both brain regions, and K_d was not affected by age.

In the frontal cortex, S-2A sites decreased in density with age (n=19; r=-0.65; p<0.01), while S-2B sites did not change. The S-2A site was the predominant subtype in this brain region with 60% more sites on the average than the S-2B subtype. Within the frontal cortex, total S-2, S-2A, and S-2B binding correlated with the S-1 density (r = 0.472, 0.514, and 0.504 respectively).

In the hippocampus, the S-2A sites also declined with age (n=21; r=-0.46; p<0.05), and as in cortex, there was no change in S-2B binding. However, the hippocampus contains twice as many S-2B sites as S-2A. This preponderance of the age stable S-2B subtype in the hippocampus may explain why the total S-2 site fails to change with age in this region. No correlation with S-1 binding density was obtained for the hippocampal S-2 site or its subtypes. In addition, hippocampal S-2 binding was not correlated with frontal cortical S-2 sites.

The levels of 5-HT and 5-HIAA did not show any age related trends in either frontal cortex or hypothalamus.

This work was supported by grants to CEF from NIA (AG-00117, AG-00446, AG-03272). DGM was supported by grants from the Glenn Foundation and NIA (AG-00093).

271.16 DISTRIBUTION OF NEURITIC PLAQUES IN THE CORTEX OF AGED RHESUS MONKEYS. R. G. Struble, L. C. Cork*, D. L. Price, Jr.*, D. L. Price and R. T. Davis*. Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205; Dept. of Psychology, Washington State Univ., Pullman, WA 99164.

Neuritic plaques (NP) occur in aging and Alzheimer's disease and, in the latter, correlate with the severity of dementia and magnitude of cortical cholinergic deficits. NP are composed of enlarged neurites surrounding a core of amyloid. It has been suggested that NP evolve from a neurite-rich, immature stage to a neurite-poor, amyloid-rich end stage (Wisniewski, H.M. and Terry, R.D., Progress in Neuropathology, Vol. II, pp 1-26, 1973).

Aged nonhuman primates are an important model for testing hypotheses about the evolution and significance of NP. In this study of six rhesus monkeys, aged 23-31 years, who had been behaviorally tested for many years, NP were counted in ten formalin-fixed hemisections of brain per animal. The types and distributions of NP were mapped using an XY plotter.

In prefrontal cortex, plaque densities varied from 0.03 to 2.79/mm^2, while in midportions of neocortex, they varied from 0.001 to 0.85/mm^2. Caudal levels had fewest NP. End-stage plaques varied from 27% to 73% with higher plaque densities associated with a high percentage of end-stage plaques.

We conclude that: 1) in animals with few NP, the immature form of NP predominates; 2) in areas of low density, NP appeared in clusters but, as plaque densities increased, clustering was obscured; 3) NP were not evenly distributed throughout various cortical regions (e.g., prefrontal and temporal cortex, particularly the inferior portions, had high densities, while area 17 showed low densities). The amygdala and hippocampus had high and low densities, respectively. This preferential distribution for NP in aged monkeys shows parallels with those seen in human brain.

Statistical analysis of these monkeys' performance on a delayed response test did not show significance between the group of 31-year old animals and their progeny (23-26 years old). However, comparison of NP densities to performance within each animal disclosed that the 26-year old monkey, which performed poorly on the test, had plaque densities comparable to those in the older group. These data suggest that, on this test, plaque densities, rather than chronological age, are better predictors of abnormalities of performance.

271.17 DENDRITIC PLASTICITY IN AGING CAT AUDITORY CORTEX: GROWTH IN COLONY CATS, REGRESSION IN CASTRATED PETS. Arnold B. Scheibel and Anne S. Kaplan. Brain Research Inst., U.C.L.A., L.A. CA 90024.

We have previously reported finding greater total dendritic length (TDL) in auditory cortex neurons from young (YP) but not old (OP) pet cats, when compared to young colony (YC) cats (Kaplan and Scheibel, Soc. Neurosci. Abst. 1981). This suggests that the relatively enriched environment in which pet cats live, and/or their peri-pubertal castration, may have a positive effect on dendritic extent, which is reversed by moderate old age.

We next added an old colony (OC) group, expecting its TDL to be at least as low as that of OP and YC. All pets (both sexes) were neutered. Old cats were 10 to 18 years of age. Basilar dendrite systems of pyramidal neurons from Layer III of the midectosylvian gyrus were drawn. Five neurons from each of five cats from each group were chosen by strictly random criteria, drawn with a drawing tube and measured on a computer graphics tablet.

The results for TDL and its two main factors, mean branch length and total number of branches, are shown below. Rather than having the least TDL, OC equalled YP in these measures. Two-way ANOVA showed no age or environment effect (p>.60), but a highly significant interaction (p= .0001) between the two variables. Post hoc t-tests between OP and OC, YP and YC, OP and YP, and OC and YC were also all significant (p<.05). Thus, aging had opposite effects in the two environments.

The 28% increase in TDL in group YP, relative to group YC, resulted from both more branches per dendritic tree (+12%), and longer bifurcating (+16%) and terminal (+14%) branches, with no change (+1%) in number of trees per cell. For group OC, on the other hand, the same TDL increase was a function of 13% more trees per cell, and of a 20% increase in lengths of terminal branches, with no changes in the number of branches per tree (+1%) or length of bifurcating branches (-3%). Thus, the patterns of dendritic growth differed between young pets and old cage raised cats.

Finally, group OP did not differ from YC by more than 10% on any measure. OP had fewer (-14%) and slightly shorter (-6%) branches than YP, suggesting dendritic dying back. OP also had far greater variance than OC for many measures. Whether such differential aging is related to the castration of the pets, or to some other variable (e.g. nutrition), remains to be determined.

Supported by USPHS grant AG01754 and AG01428.

	Young Cage	Young Pet	Old Cage	Old Pet
Total Dend. Length	2424 (103)	3111 (129)	3098 (56)	2533 (167)
Number of Branches	55.0 (3.0)	61.8 (2.6)	61.9 (1.6)	53.5 (4.2)
Mean Branch Length	44.7 (2.8)	51.0 (1.3)	50.8 (2.0)	48.0 (2.1)

Mean (s.e.m.) per cell. Lengths in microns.

271.18 MORPHOLOGICAL CHANGES IN THE TASTE BUDS OF THE ELDERLY. L.Gaitan*,G.Espinosa*,J.Espinosa* and J.P. Machado-Salas. Lab.Neuromorfologia.Neurociencias.UIICSE,ENEP Iztacala. UNAM.Tlalnepantla.Edo. de Mexico.54030.MEXICO.

Decline of sensory functions is commonly seen in the elderly.Previous studies have shown changes in the architecture of the central nervous system.Some of these changes can account for some of the sensory modifications of aged people.Nevertheless, the extent to which they participate is unknown since the anatomofunctional changes shown by peripheral structures have not been exhaustively studied.We have undertaken a series of studies aiming to approach this issue.

In this report, we describe the morphological changes observed in the taste buds of the elderly.Samples from:a) tip of the tongue, b)lingual posterolateral region, and c) lingual "V" region, were promptly removed from recently dead subjects.The age of the experimental group was over 65 years, and that of the control group between 20 and 50 years.Subjects with oral pathology or trauma were not considered for this study.All the samples were frozen-cut and processed with:Nauta-Gygax, Cajal's reduced silver nitrate and Gallego's trichromic methods. The aging human taste buds were characterized by:1)loss of the usual barrel-shaped silhoutte, which became substituted by s-shaped, cone-shaped or kinked forms, 2)degenerative changes of the cytoplasm and nuclei, of both, sustentacular and gustatory cells, 3)the taste pore region was frequently distorted and sometimes misoriented,4)also, some clear-cut degenerative changes were seen at the initial portion of the afferent nerve fibres, and 5) our statistical data have shown that the vertical diameter of aging taste buds tends to be longer than those of younger subjects; paradoxically, the depth at which the taste pore is located in the elderly is larger than that of controls. Finally, we found that the number of sustentacular and gustatory cells is similar in both groups.

It is our impression that these changes do have an important role in modifying taste perception in old age.We shall discuss some of the putative involved mechanisms.

This work was partly supported by the "Premio Sandoz" of the Academia Nacional de Medicina, conferred to one of the authors (J.P.M-S.).

272.1 STRIATAL DOPAMINE RECEPTOR SUPERSENSITIVITY AND THE ALTERATION OF MOTOR BEHAVIORAL DEFICITS IN THE SENESCENT RODENT, J.A. JOSEPH, S. SEASACK*, R.T. BARTUS, D. CLODY*, +D. MORGAN*, AND +C. FINCH. CNS BIOLOGY, LEDERLE LABS, PEARL RIVER, N.Y. 10965 +Dept. of Physiology & Biophys, USC Med. Sch., Los Angeles,CA 90033.

Previous reports (Joseph et al Life Sci., 29 575, 1981; Joseph et al Aging, 22, 1983) have indicated that the induction of development of striatal dopamine (DA) supersensitivity in senescent animals with 6-hydroxydopamine lesions or prevention of striatal DA receptor loss via dietary restriction retards age-related declines in rotational behavior, suggesting that alteration of striatal DA receptor number may be important in influencing motor behavioral performance in senescence. The present study extended these findings to other motor behaviors. Young (6-8 mo; n=7) Middle (15-18 mo, n=10) and Old (25 mo; n=10) Fisher 344 rats were implanted sc. with 2 Alzet osmotic pumps containing either vehicle (0.5N HCL)or haloperidol (.12 mg/ul in 0.5 N HCL 2.88 mg/day/pump). Pumps were left in place for 14 days, removed and 3 days later the animals were given a battery of 4 motor tests (horizontal rod walking; wire hanging; plank walking on wide medium, and narrow planks; hanging on screen inclined at 60°). Tests were carried out sequentially and later the same day the animals were given 0.5 mg/apomorphine ip. and 10 min later stereotypy assessed for 10 min (sniffs, licks, grooms, and cage crosses) Animals were sacrificed one day later, striata dissected and DA receptor activity assessed using [^{3}H]spiperone binding with domperidone (1 uM) used to determine non-specific binding. Six concentrations of [^{3}H] spiperone were used per analysis (25 to 800 p moles). Results showed that there were age differences in the vehicle treated animals on the inclined screen ($P < .05$ Kruskal Wallis Analyses of Variance) rod walk ($P < .001$) wide plank ($P < .001$ and medium plank ($P < .005$, with old animals showing significantly smaller scores (times) than young animals ($P < .05$ (Mann Whitney tests). Analyses of the difference scores computed between pairs of vehicle-treated and haloperidol treated animals revealed no age-related group differences on any of the motor tasks, indicating that all animals treated with haloperidol and withdrawn were able to show equivalent performance regardless of age on the 4 tasks. These findings were reflected in the binding data which showed that although there was a trend toward lowered receptor binding as a function of age ($P < .05$; Bmax Y = 451 ± 27, M = 420 ± 34, O = 393 ± 28 f moles/mg protein) no such trend was seen in haloperidol treated animals Y = 613 ± 36, M = 471 ± 18; O = 502 ± 35f moles/mg protein; haloperidol enhancement; Y = 36% M = 12%, O = 28%). Stereotypy scores showed enhancement in both old and young animals (e.g., grooms vehicle Y = 0.80 ± 0.80; O = 1.42 ± 0.60; haloperidol Y - 9.55 ± 4.71; O = 11.57 ± 8.17 $p < .05$ within age Mann Whitney test comparisons).

272.2 INTRACEREBRAL GRAFTING IN THE AGED RAT BRAIN. F.H. Gage, A. Björklund, U. Stenevi*, S.B. Dunnett[1]*. Department of Histology, Lund University, Lund, Sweden. Department of Experimental Psychology, Cambridge University, Cambridge, England[1].

Recent data suggest that impairments in dopaminergic and cholinergic neurotransmission, respectively, may contribute importantly to the decline in sensorimotor and cognitive function associated with the aging process. We have previously shown that lesion-induced impairments of dopaminergic and cholinergic transmission in young rats can be partially reversed both biochemically and behaviorally by grafts of embryonic dopaminergic or cholinergic neurons, and recent reports suggest that neuronal transplants can survive also in aged rat brain. In the present study we report that mesencephalic dopamine neurons and septal cholinergic neurons, taken from rat embryos, can be grafted with excellent survival to the neostriatum and hippocampus, respectively, in the brains of aged rats, and that the intrastriatal dopamine-rich transplants can ameliorate the impairments in motor coordination seen in these animals. The study employed female rats 21-23 months of age and young adult controls 2-3 months of age at the beginning of the study. One group of old rats received dissociated cell suspension grafts of embryonic septum bilaterally into the dorsal and ventral hippocampus while another group received grafts of embryonic substantia nigra bilaterally into the caudate-putamen. Behavioral testing was conducted on all rats immediately prior to and again 11-14 weeks following transplantation surgery. After the final behavioral test the animals were taken for histochemical evaluation. Surviving grafts were found on both sides of the brain in all grafted animals. Graft survival rate, volume, and histological appearance were more variable and selectively different from those previously observed in young adult host, but extensive outgrowth and cell survival were clearly apparent. Additionally the intrastriatal DA grafts were associated with a significant improvement in motor coordination abilities in the aged rats. These results suggest that the intracerebral grafting technique may provide a new tool for the exploration of the role of dopaminergic deficits in neurological and behavioral impairments associated with aging.

272.3 ACETYLCHOLINE RECEPTOR BINDING CHARACTERISTICS AT THE NEUROMUSCULAR JUNCTION OF MATURE ADULT AND AGED RATS. Michael R. Chapman and Dean O. Smith (SPON: D. Gilboe). Department of Physiology, University of Wisconsin, Madison, WI 53706.

At the rat diaphragm neuromuscular junction, there is an age-related increase in the number of nerve terminals and a corresponding rise in the amount of transmitter released. To determine if these changes are associated with altered ACh receptors, the binding characteristics were assayed using ^{125}I-labeled α-bungarotoxin. After iodination via the chloramine-T method, the labeled toxin was chromatographed on columns of Sephadex G-25 and C-25 to obtain the pure monoiodinated form. The specific activity was then determined by competition binding using solubilized Torpedo ACh receptors. Whole muscle samples from Fischer 344 rats aged 10 and 28 mos were incubated in labeled toxin. When the toxin concentration was 0.8 nM, similar to K_D, the time to half-maximal binding was 1 hr for both age groups. For the concentration ranges studied, maximal binding was attained after 2 hr at 37°C. Therefore, subsequent saturation experiments involved 2-hr incubations at 37°C in labeled toxin concentrations ranging from 0.1 to 30 nM. Binding was then assessed by Scatchard analysis. The average (±s.e.) values of B_{max} were 6.3 (±1.1) and 15.6 (±2.3) fmol/mg protein in the 10- and the 28-mos animals, respectively. Furthermore, the average (±s.e.) values of K_D were 1.1 (±0.12) and 2.7 (±0.16) nM in the younger and the older rats, respectively. Both of these age-related differences were highly significant statistically (0.01 level). Thus, the aged animals appear to have significantly greater numbers of receptors which exhibit a lower binding affinity. Supported by NIH grants AG01572 and NS00380 (R.C.D.A. to D.O.S.).

272.4 SELF-STIMULATION IN AGED RATS: EFFECTS OF AMPHETAMINE AND HALOPERIDOL. E. S. Valenstein & H. Altman. Dept. Psychology, Univ. of Michigan, Ann Arbor, MI 48109 and Lafayette Clinic, Detroit, MI 48207.

Sprague-Dawley male rats of three different age groups were tested for lateral hypothalamic self-stimulation rate at current intensities ranging from below threshold to supra-maximal levels. No differences in response rate or reward threshold was found between young (3-4 months), mature (8-9 months), and old (22-25 months) animals. When tested following injections of amphetamine (1 mg/kg) young animals displayed the anticipated increase in self-stimulation rate, but the rate of old animals tended to decrease. The response of mature animals given amphetamine was intermediate. When tested following injections of a relatively low dose of haloperidol (.06mg/kg) the self-stimulation rate of old animals was significantly more depressed than that of the young animals. Results indicate that the reward maintaining lateral hypothalamic self-stimulation in old animals is less responsive to activation by amphetamine and more vulnerable to dopamine blockade than that of young animals. It is hypothesized that the substrate of the reward system of older animals may display less reserve capacity when challenged.

272.5 PROGRESSION OF AGE CHANGES IN SYNAPTIC TRANSMISSION AT MOUSE NEUROMUSCULAR JUNCTIONS. N. Robbins and S.S. Kelly*, Dept. Anatomy, Case Western Reserve Sch. Med., Cleveland, OH 44106.

Prior physiological studies on synaptic aging have been confined to comparisons of young and old animals (e.g. Banker, Kelly & Robbins, J. Physiol., 1983). Thus, the changes observed could be agonal, secondary to aging of other organs, or alternatively, slowly progressive through life. Therefore, the progression of age-related changes in neuromuscular function was investigated in muscles from CBF-1 mice between 7 and 32 months of age. Endplate potentials (EPPs) were recorded in extensor digitorum longus (EDL), soleus, and diaphragm muscles after neuromuscular transmission was blocked with either (+)-tubocurarine chloride (curare) or high magnesium/low calcium Krebs solutions. Between 10 and 31 months of age in EDL and soleus but not in diaphragm there was an increase in EPP amplitude with age. In soleus, the maximum increase was approximately two-fold in curare and three-fold in high magnesium solution. Increase in EPP amplitude in curarised preparations took place between 20 and 28 months of age in EDL and between 28 and 31 months of age in soleus. Indirectly elicited twitch responses were used to determine the time-course of age-related changes in sensitivity to magnesium block. Increased resistance to block appeared between 15 and 19 months of age in both EDL and soleus (in which the increase was more gradual). In magnesium-blocked preparations, increased quantum content (measured directly) accounted for the increased EPP amplitude. Spontaneous miniature endplate potential (MEPP) frequency in old soleus muscles was not sensitive to low calcium/high magnesium solutions although frequency in young soleus and young and old diaphragm was significantly reduced.

The diaphragm shows no age-related changes in the physiologic parameters measured here. In limb muscle, age-related changes in evoked transmitter release begin in mid-life and take place more rapidly in EDL than in soleus. This may be due to the difference in muscle type or to the different usage of these muscles. In both muscles, age changes appear before any significant mortality or organ pathology is evident. Decreased sensitivity of transmitter release to low calcium/high magnesium is therefore an early sign of altered synaptic transmission in aging mouse limb muscle. This work was supported by NIA AG 00795.

272.6 TIME-SERIES ANALYSIS OF MORPHOLOGICAL CHANGES AT THE NEUROMUSCULAR JUNCTION OF FUNCTIONALLY DIVERSE MUSCLES DURING AGING. J.L. Rosenheimer and D.O. Smith. Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706.

The end-plate regions of the diaphragm, a phasic muscle which remains active during the aging process, and of the extensor digitorum longus (EDL) and soleus, phasic and tonic hindlimb muscles, respectively, which most likely suffer from disuse atrophy with aging, were examined in rats. Neuromuscular junctions were located following cholinesterase staining of the end plate and silver-gold impregnation of the axon and its terminal arborization. The number of terminal branches and the frequency of occurrence of sprouting and degeneration were then recorded. The mean values of 60 measurements from 5 animals of each age group (in 3-mos intervals) ranging from 10 to 31 mos were obtained. Nonlinear data smoothing techniques, according to the methods of J.W. Tukey, were applied to analyze trends. Changes in branch number were examined and compared with relative changes in sprouting and degenerating terminals.

At the diaphragm neuromuscular junction, a pronounced increase in terminal branch number was observed between 16 and 25 mos of age. Consistent with this observation, the number of sprouting terminals remained greater than the number of degenerating terminals throughout. However, sprouting decreased steadily, while degeneration increased until approximately 19 mos of age, when these trends appeared to reverse. Nerve-terminal branching within the EDL end plates decreased steadily until approximately 25 mos. The occurrences of sprouting and degeneration likewise declined with age. Sprouting remained slightly ahead of degeneration until about 25 mos of age, when degeneration was observed more frequently. Soleus neuromuscular junctions exhibited a steady decrease in branch number until approximately 19 mos; then the trend reversed, and a steady increase was seen. Accordingly, both sprouting and degeneration decreased at nearly the same rate until 19 mos. This was followed by a slight increase in sprouting through 31 mos of age. In general, these age-related changes were least pronounced in soleus, the tonic muscle. Conversely, they were more extreme in the phasic muscles. However, the number of terminal branches may be influenced by the relative amount of activity. Supported by NIH grants AG01572 and NS00380 and an NIH training grant to the Neurosciences Training Program.

272.7 TRANSMITTER RELEASE FROM CYTOPLASMIC AND VESICULAR SOURCES AT THE NEUROMUSCULAR JUNCTION OF MATURE ADULT AND AGED RATS. Dean O. Smith. Department of Physiology, University of Wisconsin, Madison, WI 53706.

At the diaphragm neuromuscular junction, there is an age-related decrease in transmitter (ACh) per nerve terminal and a corresponding increase in the number of nerve terminals per end plate. To assess correlated changes in synaptic responses, transmitter leakage and release was measured at the neuromuscular junctions of rats aged 10 and 28 mos using electrophysiological and biochemical techniques. Leakage of ACh from cytoplasmic sources was determined by measuring the hyperpolarization following application of d-tubocurarine. The hyperpolarizing responses were of similar magnitude (1 mV) in both age groups; however, the older rats were less sensitive to ACh, indicating that cytoplasmic leakage was greater in the 28-mos animals. Miniature end-plate potentials were significantly smaller in the older rats; they also occurred more frequently in the aged animals, but this increase could be attributed to the increased terminal number. In curarized preparations, end-plate potentials were recorded intracellularly during repetitive stimulation at rates ranging from 5 to 50 Hz. Synaptic depression was observed to be more extensive in the older animals. In both age groups, depression could be described quantitatively by models based strictly on depletion of transmitter. Estimates of release probability, p, were derived from these data, and the average values were 0.17 and 0.16 in the young and the old rats, respectively. As these values were significantly greater than zero, quantal release could be described using binomial statistics. Average quantal content, m, was 75 and 116 in the 10- and the 28-mos rats; this difference is significant at the 0.05 level. However, the corresponding average values are 6 and 7 per nerve terminal, and this difference is not significant statistically. Furthermore, the binomial parameter n was estimated to be 444 and 817 in the young and the aged rats, respectively; when normalized for the number of nerve terminals per end plate, however, the values were 34 and 47, which are not significantly different (0.17 level). Biochemical assays revealed that 3.2 and 3.9 fmol of ACh were released per end plate in response to 1000 presynaptic action potentials in the young and the older rats, respectively; this difference is significant at the 0.05 level. The corresponding values per nerve terminal were 0.24 and 0.22 fmol, which are not significantly different. Furthermore, in the aged animals this represents a significantly larger fraction of the total ACh present in the nerve terminals. These results indicate that an age-related increase in transmitter release may be attributed to greater numbers of nerve terminals within the end plate. Supported by NIH grants AG01572 and NS00380.

272.8 SELECTIVE REDUCTION IN THE HIGH AFFINITY OPIATE BINDING SITES IN THE PEDAL GANGLIA OF MYTILUS EDULIS DURING AGING. A. Chapman*, G. B. Stefano, M. Leung* and R. Martin[1]*. Depts. Biological Sciences and Chemistry, SUNY/College at Old Westbury, New York 11568 and [1]Electron Microscopic Section, Univ. Ulm, D-7900, West Germany.

It is expected that if specific age-related changes in neuronal function occur, these changes will be reflected in and possibly due to changes in specific neurotransmitter or neuromodulator systems and/or in other neurochemical properties. The characteristics of opioid receptors were studied in pedal ganglia (Pg) membrane suspensions of 1,2, and 4-year-old animals using both ^{3}H-etorphine and ^{3}H-naloxone. Specific and saturable high and low affinity binding was demonstrated for all age groups. When the binding data were analyzed by Scatchard plot the K_D values (high affinity 3.1nM; low affinity-11.2nM) were quite similar, however the high affinity site receptor density in older organisms was significantly reduced (young 51.0 pmol/g of protein ; old 38.0) while the density of the lower affinity sites did not change (163 pmol/g of protein). Examination of the enkephalin-like immunoreactivity in the Pg of young (1-yr) and old (4-yr) organisms revealed identical localization, however the enkephalin immunoreactivity was not as pronounced in older organisms. In an effort to resolve this observation the Met/Leu enkephalin and Met-enkephalin-Arg-Phe levels were estimated by HPLC analysis (Beckman 334, 210 sample injector, C RIA integrator on a Brownlee RP-300 column). In previous studies these opioids were isolated and sequenced from M. edulis Pg acid extracts. The levels of these opioids in this preliminary evaluation were found not to vary with age. In conclusion the high affinity opiate density appears to be selectively reduced in this organisms Pg during the aging process. Other age-related decreases in opioid regulation of dopamine levels in M. edulis have been described earlier. The significance of this selective reduction in high affinity opiate binding densities in older organisms has yet to be determined, however, such studies are important for our understanding of biological continuity, diversity and evolution.

Supported by NIH-MBRS Grant RR 08180

272.9 A GOLGI STUDY OF SUPRAOPTIC NUCLEUS TRANSPLANTS IN THIRD VENTRICLE OF YOUNG AND OLD RATS. Anne S. Kaplan, Paul D. Coleman, and Don M. Gash. Department of Anatomy, University of Rochester Medical Center, Rochester, N.Y. 14642

The supraoptic nucleus (SON) was dissected from 17-day F344 rat fetuses, and transplanted into the third ventricle of 5- and 25- month old male F344 rats. The hosts were killed 3 months later and their brains were stained by the Golgi-Cox method and sectioned coronally. Every third section was Nissl-counterstained.

The transplants were found in varying positions within or along the ventricle. The host-transplant border was sometimes distinct with a ventricular wall or capsule, and sometimes blurred, which suggests that *the two tissues had become structurally integrated.*

Nissl-stained sections showed that some but not all cells in the transplant were SON-like in appearance. Cells tended to cluster in the center or along one side of the transplant.

Golgi-stained somata varied widely in location, shape and size, but had many characteristics typical of SON neurons (see Flood and Coleman, this meeting). Their processes could usually be differentiated into dendrites and axons. A typical cell had 2 or 3 dendrities, with 0 to 3 bifurcations, for a total of 2 to 8 branches. Somata and dendrites often had long, hairy thorns. Branches from the same cell or even the same branch point differed greatly in their thickness and morphology. Many branches were studded with varicosities, which were often numerous and large. Dendrities sometimes ended with a varicosity, or with a bristly, diffuse, wide mass, suggestive of a growth cone.

The path of some of these processes was very tortuous, akin to those seen in normal SON. Many however, especially those running in cell-poor areas of the tissue, were quite direct. These tended to be much longer than normal SON dendrites. Processes from cells in or near the wall or capsule tended to stay there, running in parallel, or to turn to enter the donor tissue. Some even turned to enter the host tissue. Dendrities originating from cells within the center of the transplant tended to head towards the ventricle floor (median eminence) or towards ventricle or capsule walls. There, most either terminated or turned to follow the wall, but some, again, penetrated the graft-host interface, projecting into the host parenchyma. In addition, some host cells sent processes to or through the transplant wall, although most host fibers in the vicinity of the graft simply ran alongside it.

In summary, both old and young host rats were able to support hypothalamic transplants, with cells that appeared quite robust and normal in form. Further, there was some evidence of two-way anatomical interaction between host and transplant tissues.

Supported by the National Institute on Aging (AG 1121) to P.D.C. and NIH- NS 15109 to D.M.G.

272.10 DENDRITES OF DENTATE GRANULE CELLS AND HIPPOCAMPAL PYRAMIDAL NEURONS IN HUMAN AGING AND DEMENTIA. S.J. Buell*, C.H. DeFiore, G.J. Horwitz* and P.D. Coleman. Dept. Neurology, Univ. Rochester Sch. Med. & Dent., Rochester, NY 14642.

We have extended our finding of dendritic growth in layer II pyramidal neurons of the parahippocampal gyrus in normal human aging but not in aging with dementia (Buell & Coleman, Science 206:854, 1979) by assessing dendritic extent in the granule cells of the dentate gyrus and pyramidal neurons of sector H-1 (approximately CA1) of Ammon's horn. From over sixty cases obtained at autopsy, five normal adults (avg. age=51 yr.), five aged cases (avg. age=79 yr.) and five cases with senile dementia of the Alzheimer type (avg. age=76 yr.) were selected. All cases were free from significant neurological or psychiatric disorder, except the cases in the latter group had a well documented history of late stage Alzheimer's disease. All cases were also free from significant neuropathology at autopsy, except that the cases in the latter group had abundant senile plaques and/or neurofibrillary tangles in the hippocampus and adjacent neocortex. Neurons were selected randomly for analysis from coded 200um thick sections of tissue prepared by the Golgi Cox method. The groups did not differ significantly in post mortem time (PMT) which averaged 12 hrs. Dendritic extent tended to be greater in the aged than the adult or demented cases in both the granule cells of the dentate gyrus and the pyramidal cells of Ammon's horn. Dendritic trees of demented cases were slightly smaller than those of the adults. Differences among groups tended to be greater in terminal than in non-terminal segments and were largely confined to the middle range of somatofugally-ordered segments. The magnitudes of the differences among groups were nearly identical in these two cell types and are very similar to the group differences we saw previously in layer II pyramidal neurons of the parahippocampal gyrus. Analysis of covariance showed that when total dendritic length was corrected for effect of PMT, differences among groups were not statistically significant. However, this form of analysis showed that the groups did differ significantly in dendritic extent in the middle range of somatofugally-ordered segments in the dentate granule cells ($p < 0.05$) but not in the hippocampal pyramidal neurons. Values for the aged group were significantly larger than those for the adult and demented (t-test, $p < 0.02$ and $p < 0.01$, respectively) which did not differ significantly from each other. This accentuation of differences among groups in middle dendritic orders has been a consistent finding in our studies of dendrites in aging and dementia. Supported by NIH Grant AG 02680.

272.11 DENDRITIC EXTENT OF LAYER II PYRAMIDS IN ENTORHINAL CORTEX OF AGING F344 RAT. P.D. Coleman and S.J. Buell*. Departments of Anatomy and Neurology, University of Rochester Medical Center, Rochester, New York 14642.

Previous work from our laboratories has indicated apparent dendritic growth of Golgi-Cox stained layer II pyramidal neurons in parahippocampal gyrus of aged as compared to middle aged humans (Buell and Coleman, Science, 1979; Brain Research, 1981). Interest in developing an animal model of this phenomenon led us to quantify the same cell type in the same layer in a comparable cortical area (entorhinal) of the aging male F344 rat. Tissue was stained by the Golgi-Cox method. Section thickness and random cell selection procedures from coded slides were similar to those used in our earlier human study. Dendritic trees were traced by means of a drawing tube. These tracings were quantified with the aid of an Apple II+ microcomputer with a drawing tablet. The data show no significant differences between mean dendritic measures at 12 months and 30 months. Total dendritic length per cell at 12 months was 2,189 um; at 30 months 2,205 um. However, the within animals standard deviation for total dendritic length averaged 578 um for the 12 month and 951 um for the 30 month old animals ($p<0.05$ one tailed). Increased variance in older animals is a usual finding in aging studies. Within the context of the present study this increased variance suggests that cells whose dendritic lengths depart more from the mean are more common in 30 month than in 12 month animals. To maintain the same average dendritic length in the two age groups cells with regressing dendritic trees seem to be balanced rather precisely by cells with expanding dendritic trees in the 12 to 30 month period. Data available to date suggest significant species differences in aging of cortical neurons in relation to life span as defined by survival curves.
Supported by The National Institute on Aging (AG1121 and AG2680).

272.12 AGE-RELATED CHANGES IN DENDRITIC EXTENT OF NEURONS IN SUPRAOPTIC NUCLEUS OF F344 RATS. D.G. Flood and P.D. Coleman. Dept. of Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

Dendrites and cell numbers were examined in the supraoptic nucleus of male F344 rats ranging in age from 3 to 32 months. Cells with a visible nucleolus were counted in every 20th cresyl violet stained 10 um section in rats aged 12, 20, 27, 30, or 32 months. No change was found in cell numbers as a function of age. These data extend those of Hsu and Peng (Gerontol. 24, 434-440, 1978), who studied female Sprague-Dawley rats. In rats aged 3, 12, 20, 27 or 30 months, dendritic trees of supraoptic neurons were examined in Golgi Cox impregnated tissue. Generally, the impregnated neurons were found in the posterior and superior portions of the nuclei. The neurons had dendrites that projected ventrally, seldom branching. Because of their simple structure, quantitative changes occurred only in dendritic length measures and not in measures of branching. Between 20 and 27 months of age, dendritic length was drastically reduced. No further reduction occurred between 27 and 30 months. These data suggest that dendritic atrophy is not necessarily a prelude to cell death. Between 12 and 20 months of age, Sladek et al. (Peptides 1, Suppl. 1, 141-157, 1980) have found a decline in the noradrenergic innervation of the ventral portion of the supraoptic nucleus. These data suggest that partial deafferentation may be producing the age-related dendritic atrophy seen in this system. Supported by PHS grants AG 01456 and AG 01121.

272.13 DENDRITES IN EXTREMELY OLD MOUSE CORTEX. L. Magagna*, P.D. Coleman and S.J. Buell*, (SPON: E.D. Caine). Dept. Neurology, Univ. Rochester Sch. Med. & Dent., Rochester, NY 14642.

Dendritic regression has been reported in a number of studies in which the aged animals were very old in terms of species or colony life span characteristics. Dendritic growth has been reported in studies in which aged subjects were relatively younger. We have analyzed dendritic extent in spiny stellate neurons of the posteriomedial barrel subfield of C57Bl/6NNia mice at 36 and 45 months-of-age for comparison with our earlier series of mice at 4, 12, 22, 26, and 30 months-of-age. In this earlier series we observed stability of dendritic extent throughout the 26 month portion of the life span studied (Buell et al., The Gerontologist 22:234, 1982). In both the previous and the present study 140 micron sections impregnated by the Golgi Cox method and counterstained with Cresyl violet according to Ramon Moliner were coded before analysis. Five randomly selected neurons from each of three animals at each age were studied for a total of 30 cells at 36 and 45 months-of-age and a total of 105 from 4 to 45 months-of-age. Dendritic extent was estimated from camera lucida drawings entered into a microcomputer via a digitizing tablet. Our results gave no indication of dendritic regression even at 45 months-of-age(the published 1% survival age for this colony is 33 months). Qualitatively, there was no detectable difference in appearance of the impregnated dendritic trees in any of the old animals when compared to the young. Likewise, there was no quantitative evidence for dendritic regression in any of the old animals. On the contrary, there was evidence for significant dendritic growth (ANOVA, $p<0.05$) in the middle range of centrifugally ordered dendritic segments as a function of advancing age from 4 to 45 months. Inspection of the group data revealed than mean value for each of the three oldest groups (30, 36, 45 months) was greater than the mean values of all of the four youngest groups (4, 12, 22, 26 months). We conclude that dendritic regression is not an inevitable consequence of advancing age, even in extremely old individuals. Some potential for dendritic growth appears to be preserved even at very late stages in the life span of this colony. Supported by NIH Grant AG 02680.

272.14 QUANTITATIVE ELECTRON MICROSCOPY OF DENDRITIES IN LAYERS I AND II OF ENTORHINAL CORTEX OF AGING F344 RAT. M.N. Sheridan*, T. Langow* and P.D. Coleman (SPON: V. Laties). Departments of Anatomy, Uniformed Services, University of Health Sciences, Bethesda, MD 20814, and University of Rochester Medical Center, Rochester, NY 14642

Dendrites were quantified in electron micrographs of layers I and II of lateral entorhinal cortex of male F344 rats aged 4, 20 and 32 months. Brains were fixed by perfusion with buffered aldehydes, post fixed in osmium tetroxide, dehydrated in alcohol and embedded in Epon. Electron micrographs were prepared at a final magnification of 17,500. Over 13,000 dendritic profiles were counted and measured. Dark dendritic profiles were included in the sample. Profiles too small to be positively identified as dendrites were excluded from the sample. The average cross sectional area of dendrites in layer I was 1.75 μm^2; in layer II it was 1.91 μm^2. Average circumference per dendritic profile in layer I was 5.69 μm; in layer II it was 5.70 μm. In layer I there was an average of 0.115 dendrities per μm^2 and the volume fraction of dendrites was 0.20. Analysis of variance showed no significant age-related trends in any of the measures taken. These electron microscopic data are consistent with Golgi-Cox data showing no significant age-related changes in dendritic extent in entorhinal cortex of the male F344 rat.

Supported by the National Institute on Aging (AG1121) and by USUHS Grant CO7024.

272.15 THE EFFECTS OF AGING AND EXERCISE UPON REGIONAL BRAIN GLUCOSE OXIDATION. R.P. Farrar*, C.M. Ardies*, P.E. Gilliam, H.L. Dodd* & W.W. Spirduso. Dept. of Physical and Health Education, University of Texas, Austin, Texas 78712.

As rats age there is a progressive decline in spontaneous motor activity. Concomitmant with this decline is a reduction of capillarization and blood flow in the caudate, a decline in oxidative metabolism in the brain, and a decline in reactive capacity. Previously we have measured an attenuation of the decline in reactive capacity in rats that initiated running at 18 months and continued until 24 months when compared to their sedentary controls. Due to the shifts in blood flow induced by regional brain activity it was hypothesized that a regular running regime might better maintain regional blood flow and oxidative metabolism in the caudate, motor cortex, and cerebellum. Sedentary male Fisher 344 rats 3,6,12,18, and 24 months of age were compared to rats 18 and 24 months of age that had run on a treadmill 5 days/week, 30 min/day, from the age of 3 months. The caudate exhibited a significantly greater rate of glucose oxidation, 18.94 $\pm$ 0.6 umoles of glucose/mg/30 min at 3 months, than did the motor cortex, 12.79 $\pm$ 0.53, or the cerebellum, 10.85 $\pm$ 0.54. The caudate also exhibited the greatest decline in oxidation down to 10.11 $\pm$ 1.24 umoles at 18 months compared to 7.67 $\pm$ 0.54 umoles for the motor cortex, and 6.92 $\pm$ 1.01 umoles for the cerebellum. Surprisingly, glucose oxidation did not decline from 18 to 24 months in any region and exercise did not significantly attenuate the decline in any region at either 18 or 24 months.

Sponsored by NIH GRANT RO1-AG-02071-01A1

273.1 SLOWLY DEVELOPING HYPERRESPONSIVENESS OF CRAYFISH NON-GIANT ESCAPE CIRCUITRY FOLLOWING NERVE CORD TRANSECTION. M.T. Lee and J.J. Wine. Dept. of Psychol., Stanford Univ., Stanford, CA 94305.

In intact crayfish, gradually applied tactile stimulation of the abdomen can evoke a series of tailflips, i.e., alternating, rapid flexions and extensions of the abdomen. The production of this behavior does not involve the giant axons (MG and LG); instead, it appears to be dependent upon activity in other axons that travel in the dorsal part of the nerve cord. Transection of the cord below the subesophageal ganglion immediately abolishes "non-giant" tailflips. The cord does not regenerate across the lesion, and normal non-giant tailflips never return. However, sensory-evoked activity in the abdominal fast flexor (FF) muscles develops within a few weeks. We have begun to analyze the cellular basis of this return of function.

The nerve cords of crayfish (Procambarus clarkii) 7-11 cm in length were transected completely between the 5th thoracic and 1st abdominal ganglia. At various times after the operation, abdominal nerve cords were isolated along with the tailfan, which was left attached to the 6th ganglion via its nerve roots. In acutely transected preparations, intracellular recordings from the FF motoneurons reveal only very small depolarizations in response to tactile stimulation of the tailfan. By 7 weeks after transection, these same stimuli produce intense barrages of EPSPs, sufficient to evoke copious spikes in many of the FF motoneurons. Although spike output varies greatly among cells, increased responsiveness has been found in all FF motoneurons examined in abdominal ganglia 2-5 of chronically transected preparations. During flexor bursts, the telson flexor motoneurons and the peripheral inhibitor of the fast extensor muscles also burst strongly. In contrast, the fast extensor motoneurons are silent, and the fast flexor inhibitor fires either weakly or not at all. Moreover, elements restricted to the giant escape circuitry (MG, LG, segmental giant, and motor giant) are also silent. These results suggest that changes occurring within the isolated abdominal nervous system specifically enhance the excitability of the non-giant escape circuitry. The enhancement appears to be limited to the flexion portion of non-giant escape, although this may be merely the result of the method of stimulation or the type of preparation used. Further experiments are under way to determine the sites of these changes.

Supported by NIH postdoctoral fellowship NS 07033-01 (M.T.L.) and NSF grant BNS 81-12431 (J.J.W.).

273.2 NEONATAL 6-OHDA TREATMENT OF RATS: CHANGES IN DOPAMINE (DA) RECEPTORS, STRIATAL NEUROCHEMISTRY AND ANATOMY. Richard B. Mailman, Andrew Towle, David W. Schulz, Mark H. Lewis, George R. Breese, Diane L. DeHaven and Martin R. Krigman. Biol. Sci. Res. Center, Depts. of Psychiatry, Pharmacology and Pathology, University of North Carolina Sch.of Med., Chapel Hill, NC 27514.

Within 18 hrs of birth, Long-Evans rats were given an intracisternal injection of 6-hydroxydopamine [(OHDA) 30mg/10 mL ascorbate-saline] 30 min after 20mg/kg DMI, and then sacrificed at 45-50 days of age. Three separate experiments were performed with N_{6OHDA} = 13, 4, 3 rats, and $N_{control}$ = 10, 4, 3 rats, respectively per experiment. The striatum was dissected into two regions. That part of the caudate putamen in a coronal section ca. 1.6 -2.6 mm anterior from bregma formed what is termed "striatum". All rostral portions of the striatum were termed "head of the caudate". In the "striatum", there was a significant decrease in neuropil, as well as in the total number of synapses. Moreover, there were very few remaining fibers with tyrosine hydroxylase immuno- reactivity (TH-IR). However,the binding of [^{3}H]-dopamine (5nM) was decreased by 62%, 50%, and 67% for the three experiments, respectively. Interestingly, there were no detectable changes in either [^{3}H]-spiperone binding, or in the sensitivity of dopamine stimulated adenylate cyclase. In the same animals, the concentrations of DA in the "head of the caudate" was decreased by 98%, 98% and 99%, whereas the concentration of 5-hydroxytryptamine (5-HT) was increased by 62%, 50% and 67%, in the three experiments respectively. The latter finding confirms earlier work noting that neonatal 6-OHDA treatments significantly increase 5-HT in this area of the striatum (Stachowiak et al., Soc. Neurosci. Abstr. 8:304, 1982). It is presently unknown what the exact nature is of the binding sites for spiperone or dopamine, although dopaminergic agonists and antagonists clearly have markedly different affinities for those binding sites that are common. In the present experiments, the persistent (i.e., for at least 45 days after treatment) decrease in [^{3}H]-dopamine binding occurs in the absence of any changes in the binding of [^{3}H]-spiperone, or in the sensitivity of adenylate cyclase to dopamine. This suggests that the loss of [^{3}H]-dopamine binding sites are predominately from populations other than the ones normally designated as D_1. Similiar treatments to those used here cause profound "receptor supersensitivity" (i.e., apparent increase in behavioral potency of dopamimetic drugs), yet no "receptor up-regulation" was detected in these experiments. The reasons for this are certainly relevant to the general question of the physiological meaning of changes in receptors as measured by radioligand binding.

(Supported in part by ES01104, HD/MH16834 and HD03110.)

273.3 DEVELOPMENTAL CHANGES IN THE NEURAL CIRCUITRY FOR TOUCH SENSITIVITY IN CAENORHABDITIS ELEGANS. M. Chalfie[1], J.E. Sulston[2*], J.G. White[2*] and J.N. Thomson[2*]. [1]Department of Biological Sciences, Columbia University, New York, NY 10027, [2]MRC Laboratory of Molecular Biology, Cambridge, England.

We have used selective cell killing and reconstruction from serial section electron micrographs to analyze the neural circuitry involved in touch-mediated movement in the nematode Caenorhabditis elegans. This circuitry is modified during C. elegans development by the addition of cells and the loss of specific synapses. For both the anterior touch response (resulting in backward movement) and the posterior touch response (resulting in forward movement) the pattern of synapses is similar: the touch cells form gap junctions and apparent chemical synapses to the major ventral cord interneurons. These, in turn, synapse onto the motor neurons of the ventral cord. Using laser microbeam killing of cells of each of these classes, we have determined that the gap junctions are the most important synapses for touch-mediated movement. Thus, touch receptors in the tail form gap junctions to interneurons (the PVCs) which in turn synapse onto motor neurons that make the animal move forward. In the young larvae a similar circuit occurs in the head: the touch cells form gap junctions to interneurons (the AVDs) that synapse onto motor neurons that make the animal move backward. However, midway through larval development, a postembryonic touch cell (AVM) sends branches to each of the pre-existing touch cells in the head (ALML and ALMR) and forms gap junctions with each. AVM appears to serve two functions: 1) it joins with ALML and ALMR to form a nerve net of touch receptors (this may be required in C. elegans because the cells do not have intricate branching patterns and 2) it provides this nerve net with chemical synapses onto an additional class of interneurons, the AVBs. These AVB synapses appear to be important since in adults the gap junctions between these touch cells and the AVD interneurons are lost. As a result of this loss, animals which lack AVM (by prior laser killing) become touch insensitive in the head 3-4 days after becoming adults. The loss is quite stochastic: in nine experimental animals, some were virtually touch insensitive, others were almost wild type. This loss is quite specific since both the touch cells and the AVD interneurons retain gaps junctions with other neurons, and, in the case of AVD form additional gap junctions to other neurons. These changes in the touch circuitry appear to be part of what may be extensive respecification of the C. elegans nervous system during development.

273.4 PROPERTIES OF GLUTAMIC ACID DECARBOXYLASE IN HOMOGENATES OF CRAYFISH CNS TISSUE AND EFFECTS OF CRUSH ON ENZYME ACTIVITY. R.M. Grossfeld, S.W. Yancey* and D.B. Hansen*. Zoology, Dept., North Carolina State University, Raleigh, NC 27650.

Glutamic acid decarboxylase (GAD) activity is maintained for 2 weeks in isolated peripheral nerves of crayfish despite the coincident loss of CAT and AChE activities (Sarne et al., Brain Res. 110:91-97, 1976). Our goal was to examine the stability of GAD activity in isolated CNS tissues of crayfish.

GAD activity was measured in homogenates of crayfish abdominal nerve cord. Our procedure reliably estimates GAD activity and yields data consistent with that of Wu et al. (Trans. Am. Soc. Neurochem. 7:190, 1976). GABA and CO_2 were produced in roughly equimolar amounts. Product formation was linear for 10 h. at 11-32° and had an optimal pH of 7-10. There was no significant catabolism of product. Decarboxylation of L-glutamate was inhibited 35% by 5 mM D-glutamate or α-ketoglutarate but less than 12% by substrate analogs of varying chain length. There was an optimal concentration for stimulation by pyridoxal phosphate, 2-mercaptoethanol, or potassium phosphate. Sodium phosphate altered the stimulatory effect of potassium phosphate. Inhibition by the carbonyl trapping agent aminooxyacetic acid (AOAA), the sulfhydryl reagent DTNB, the product GABA, or chloride was competitive with substrate. AOAA also competed with coenzyme. Product analogs of varying chain length were ineffective inhibitors. SCN^{-1}, I^{-1}, Zn^{+2}, and Mn^{+2} were the most potent inhibitory ions. Acetate, F^{-1}, and phosphate were stimulatory. EDTA, Mg, NAD, and nicotinamide had no significant effect. GAD in homogenates of crayfish CNS tissue behaves like a typical neural GAD I rather than like the non-neural GAD II, but is distinct from GAD I of other species.

We examined GAD activity in CNS connectives and ganglia which had been crushed at each end to isolate them in vivo from central and peripheral connections, and in comparable tissues from sham-operated control animals. The lesions produced about a 30% decrease in the total and specific activities of GAD in the isolated connectives and ganglia during the first 2 post-operative weeks; however, there was little or no change during the ensuing 6 weeks. During this time, there was no apparent recovery of function. GAD activity appears to be conserved for at least 2 months after injury of crayfish CNS tissues, even in those isolated tissues (connectives) in which there are no known nerve cell bodies. (Supported in part by Grant 2036 from N.C. Board of Science & Technology and by N.C. Agricultural Research Service Project 5490).

273.5 DENERVATION-INDUCED ELEVATION OF ENDPLATE BAND ACETYLCHOLINE RECEPTORS. T.A. Levitt-Gilmour and M.M. Salpeter. Section of Neurobiology & Behavior, Cornell Univ., Ithaca, NY 14853.

Studies using gamma counting reveal that the number of acetylcholine receptors within the endplate band doubles by 2 days after denervation (C.C. Chang et al., J. Physiol. 250:161, 1975; A. Olek et al., Brain Res. 214:429, 1981; T.A. Levitt-Gilmour & M.M. Salpeter, unpublished data). By 8 days after denervation, no such increase is seen. The endplate-band label is obtained after subtraction of non-endplate band counts on a per weight basis, assuming a uniform distribution of extrajunctional label. Therefore, the early increase in the endplate-band specific label could be due to an increase in the site density of receptors localized in the postjunctional folds, or to a non-uniform development of extrajunctional receptors after denervation.

We treated 4 day denervated sternomastoid muscle of female albino mice in one of two ways: 1) the muscle was saturated with ^{125}I-α-bungarotoxin (^{125}I-α-BGT) 4 days after denervation, or 2) the muscle was pretreated with unlabelled BGT just before the nerve was cut, and 4 days later, fixed and labelled with ^{125}I-α-BGT, thereby labelling only newly inserted receptors. For an internal control, the innervated muscle of each mouse was saturated with ^{125}I-α-BGT. All tissue was then processed for EM autoradiography. We determined the site density of receptors in 3 regions: within the junctional folds (junctional receptors); in extrajunctional membrane within the endplate band; and in non-endplate regions (which by our dissection was at least 2 mm from the endplate). The site density of junctional receptors was the same for innervated and denervated muscles. In denervated muscle pretreated with unlabelled toxin, the junctional site density was that expected from replacement of receptors degrading with a slow half-life (∿8 days). On the other hand, the site density of extrajunctional receptors within the endplate band was ∿150 sites/μm^2, and similar to that seen in non-innervated muscle (S. Bevan & J.H. Steinbach, J. Physiol. 267:195, 1977), whereas the site density in the non-endplate band was ∿3 times lower (∿40 sites/μm^2). This difference represents a gradient of extrajunctional receptors away from the junction, similar to that reported during development (Bevan & Steinbach, 1977; S. Burden, Dev. Biol. 57:317, 1977). We calculate that the increased extrajunctional site density over the area of membrane in the endplate band is large enough to account for the elevated receptor values obtained by the 4 day gamma count experiments. In conclusion, our data show that after denervation there is no increase in junctional receptors, but extrajunctional receptors first develop near the junction and by 8 days are distributed throughout the membrane, giving rise to a "spread of supersensitivity."

(Supported by NIH grant NS 09315)

273.6 BEHAVIORAL AND ANATOMICAL CONSEQUENCES OF PERIPHERAL NERVE LESIONS WITH POSSIBLE RELEVANCE TO DEAFFERENTATION PAIN. B. E. Rodin and L. Kruger. Depts. of Anatomy and Anesthesiology, UCLA Center for the Health Sciences, Los Angeles, CA 90024.

I. The hypothesis that autotomy - self-inflicted damage to a deafferented body part - is a pain-related behavior was tested by comparing the incidence/severity of autotomy in adult rats subjected to bilateral lesions of the saphenous and sciatic nerves and either (1) no additional special treatment (control) or (2) neonatal treatments that compromised the fiber systems believed to contribute to deafferentation pain. Unmyelinated primary somatosensory axons were destroyed in one group of animals by treatment with capsaicin; postganglionic sympathetic fibers, reported to play a role in ectopic impulse generation in injured sensory axons, were destroyed in a second group by treatments with 6-hydroxydopamine. The efficacy of the neurotoxins was determined by electron microscopic or immunohistochemical studies of dorsal root, spinal cord and superior cervical ganglion. Neither neonatal treatment reduced the level of post-denervation autotomy relative to control behavior, a result indicating that pain is probably not an etiologic factor in autotomy.

II. The potential reinnervation of denervated cutaneous zones with collateral sprouts from nearby intact nerves was examined in adult rats by labeling dorsal root ganglion (DRG) cells with a lectin-horseradish peroxidase (WGA-HRP) conjugate for transport labeling of cutaneous sensory fibers. The distribution of saphenous nerve sensory axons in the plantar skin of the hind paw was studied at acute (control; 2 days) or long (2 wks-6 mos) intervals following section of the ipsilateral sciatic nerve. Behavioral testing included responsiveness to hemostat-pinch of the plantar skin throughout the postoperative survival period. Since the sensory innervation of the rat hind paw originates in the L4 and L5 DRG, these ganglia were injected with WGA-HRP; two days later the animals were sacrificed and the DRG and plantar skin were reacted with tetramethyl benzidine. Control material consisted of labeled skin from the hind paw contralateral to the chronic lesion or from the ipsilateral hind paw in different animals.

Behavioral and anatomical evidence indicated that under normal circumstances the saphenous nerve projects to the medial third and the sciatic to the lateral two-thirds of the hind paw. Following sciatic lesions, the saphenous projection to lateral plantar skin gradually increased and, by 6 mos, was dense across the entire plantar surface of the paw. The nature of the sprouting fiber population is currently under investigation by re-examining sprouting in animals treated neonatally with capsaicin.

Supported by NIH grant NS-5685 and an NRS award to B.E.R.

273.7 NEURAL REGULATION OF NORMAL AND DYSTROPHIC AVIAN MUSCLE. S. Howlett* and T.B. Hoekman* (SPON: C. Harley). Basic Sci. Div., Fac. of Med., Memorial Univ. of Nfld., St. John's, Nfld, Canada A1B 3V6.

The etiology of muscular dystrophy has been variously described as either primarily neurogenic, myogenic or vascular in origin. One current view is that a generalized membrane abnormality is the primary defect and different tissues are affected to varying extents. To evaluate the neural regulatory role in avian dystrophy, the contractile responses of innervated and denervated muscle to acute close intra-arterial (IA) injections of acetylcholine (ACh) were examined in normal (Line 412) and dystrophic (Line 413) New Hampshire chickens. The in vivo nerve-muscle preparation of the avian extensor digitorum communis muscle was used (Howlett, S.E. & Hoekman, T.B. Exp. Neurol., In press, 1983) with four study groups; normal control (NC), dystrophic control (DC), normal denervated (ND), and dystrophic denervated (DD). Acute IA drug injections were administered as 30 ul volumes during continuous direct twitch stimulation. Two contractile measures were examined, the % twitch depression and the baseline contracture (% maximum twitch).

Denervation caused a 100-fold shift to the right for the ACh log dose-response curves for both twitch and contracture responses in normal and dystrophic muscle. This is consistent with the extrajunctional ACh sensitivity which develops in denervated muscle. The contracture response was significantly increased ($p<0.05$) in the ND group when compared to the DD group although the contracture response was similar in NC and DC groups. In the control group the % twitch depression was significantly greater ($p<0.05$) in NC than in DC birds and in the denervated group, the twitch response was depressed in ND when compared to DD birds. These data suggest that differences in the neural regulation of the muscle response to ACh exist in normal and dystrophic avian muscle. To determine whether these differences reflect alterations in either the receptor properties or in excitation-contraction coupling, the acute responses to IA injections of caffeine and KCl are currently being investigated.

This work was supported by grants from the Medical Research Council of Canada (MA 6477) and the Muscular Dystrophy Association of Canada.

273.8 **METHYLPREDNISOLONE PRESERVATION OF CAT MOTOR NERVE FUNCTION DURING EARLY ANTEROGRADE DEGENERATION.** E.D. Hall and D.L.Wolf, CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

Previous work has demonstrated that intensive pretreatment with the glucocorticoid triamcinolone in cats (8 mg/kg i.m./day for 7 days) acts to preserve the function of cat soleus motor nerve terminals during early anterograde degeneration (Hall et al., Ann. Neurol. 1:263, 1977; 2:404, 1977; Exp. Neurol. 79:488, 1983). The present study was undertaken to determine whether this effect of high dose glucocorticoid treatment is peculiar to triamcinolone or whether it can be duplicated by a structurally distinct glucocorticoid. Accordingly, 4 cats were treated with methylprednisolone (MP) acetate (Depo-Medrol^R) in a regimen of 8 mg/kg i.m./day for 7 days. On the 7th day, one leg was denervated by aseptic sciatic nerve section at the hip under pentobarbital anesthesia, and 48 hr later a bilateral in vivo soleus nerve-muscle preparation was performed as described in the above refs. Several neuromuscular functional parameters were examined in the 48-hr nerve sectioned and the contralateral non-nerve sectioned soleus muscles in both MP-treated and untreated (N=4) cats.

The mean ratio of the isometric contractile responses to supramaximal nerve stimulation at 0.4 Hz of the 48-hr nerve-sectioned soleus muscle to that of its contralateral non-nerve sectioned muscle was 0.2± 0.14 in the untreated cats vs 0.68±0.20 in the MP-treated animals ($p< 0.1$). Thus, MP treatment appeared to result in a 3-fold greater number of functional soleus motor units at 48-hr after nerve section. Secondly, the degree of maintenance of maximal tetanic muscle tension during brief high frequency stimulation (100 Hz/10 sec) was only 35.7± 13.1% in the untreated 48-hr preps vs 84.4±7.3% in the MP-treated 48-hr ones ($p < 0.05$). Furthermore, a post-tetanic potentiation (PTP) of contractile strength was observed in 4/4 MP-treated 48-hr nerve-sectioned soleus muscles, indicative of the occurrence of a stimulus-bound repetitive discharge (SBR) of the trophically-compromised motor nerve terminals, while SBR-PTP never occurred in the untreated 48-hr preps. Similarly, the MP-treated 48-hr degenerating motor nerve terminals were significantly more responsive to the facilitatory effects (SBR generation) of a 200 μg/kg i.v. dose of edrophonium. Finally, a 150 μg/kg i.v. test dose of d-tubocurarine produced a mean 91.3±5.4% decrease in contractile tension in the untreated 48-hr nerve-sectioned preps vs only a 26.6±8.0% transmission block in the MP-treated degenerating ones ($p < 0.001$).

In conclusion, intensive pretreatment with MP, as shown previously with triamcinolone, acts to preserve the excitability and transmitter function of degenerating motor nerve fibers, thus showing that this is a common effect of high-dose glucocorticoid treatment that may be useful in the treatment of certain degenerative neuromuscular disorders.

273.9 DEGENERATION OF PERIPHERAL NERVE IN THE RAT TRIGEMINAL SYSTEM. W. E. Renehan* and B. L. Munger* (SPON: B. Hwang). Dept. of Anatomy, College of Medicine, Pennsylvania State University, Hershey, PA 17033.

While a great deal of attention has been focused on the study of myelinated axons following a transection or crush lesion, relatively little information is available concerning the course of degeneration at the level of the peripheral axon terminal. In light of the importance of this data to both the clinician and the neuroscientist, we chose to examine sensory terminal degeneration using the highly ordered and dense innervation of the rat vibrissa. Adult Sprague-Dawley rats were subjected to transection or crush of the infraorbital nerve approximately 2mm distal to the infraorbital fissure. Animals were sacrificed at 6, 12, 15, 18, 24 and 72 hours following surgery, and perfused with a Forssmann's rinse followed by a 2% glutaraldehyde-2% paraformaldehyde fixative. Individual vibrissae were dissected from the mystacial pad, embedded in araldite (Durcupan, Fluka), and examined with a Philips 400 transmission electron microscope. The Ruffini terminals, free nerve endings (FNE's), and lanceolate-like terminals in the loose connective tissue at the roof of the ring sinus, Merkel cell-neurite complexes of the outer root sheath, lanceolate terminals of the mesenchymal sheath and free nerve endings (FNE's) below the ringwulst were monitored for evidence of degeneration. No observable changes were detected until 18 hours after surgery, at which time some large diameter myelinated axons displayed moderate to severe axoplasmic disruption and focal myelin breakdown. Occasional Merkel and lanceolate terminals evidenced axoplasmic vacuolization with mitochondrial clumping and distention. The Ruffini terminals and FNE's displayed no degenerative changes at this time. At 24 hours, virtually all the Merkel cell-neurite complexes and lanceolate receptors were deafferented. Large diameter sensory axons showed severe degenerative changes, including vacuolization of cytoplasm and myelin breakdown. The Merkel cells themselves were unchanged, but the Schwann cells associated with the lanceolate receptors contained electron-dense amorphous inclusions, presumed to be phagocytosed axon terminals. The Ruffini complexes and FNE's still displayed no degenerative changes at 24 hours. At 72 hours, however, both Ruffini terminals and FNE's were no longer present. Many of the residual Schwann cells displayed reduplication of basal lamina and contained remnants of phagocytosed myelin. We conclude that the sequence of degeneration is first observed in those receptors innervated by large diameter myelinated axons, with those supplied by small diameter myelinated and unmyelinated axons persisting for a significantly greater period of time. (Supported in part by USPHS HD-11216.)

273.10 NEUROTROPHIC REGULATION OF THE AUTOPHOSPHORYLATABLE REGULATORY SUBUNIT OF PROTEIN KINASE TYPE II (RII). S.P. Squinto*, J.A. McLane and I.R. Held. Depts. of Biochemistry and Pharmacology, Loyola Univ. Med. Center and V.A. Hospital, Hines, IL 60141.

The motor nerve transmits regulatory information to the contiguous muscle cell through the impulse-directed and spontaneous release of ACh or through the release of neurotrophic factors which are synthesized in the neuronal perikarya and delivered to the neuromuscular junction by axoplasmic transport. The molecular mechanism(s) by which the muscle cell translates these non-impulse mediated neural messages into biological responses is not known. Previously, we reported that the in vitro phosphorylation of a 56,000-dalton soluble cytosolic protein is increased early (3 hr) after denervation of the slow-twitch soleus muscle of the rat and that this phosphorylative modification is temporally correlated with the length of distal nerve stump left attached to the muscle (J. Neurosci., in press, 1983). Additionally, we have tenatively identified the 56,000-dalton phosphoprotein as RII (Proc. Int. Soc. Neurochem., in press, 1983), the autophosphorylatable regulatory subunit of cyclic AMP-dependent protein kinase. Autoradiographs of ^{32}P-labeled tryptic fragments produced by limited proteolysis of the 56,000-dalton phosphoprotein and resolved by 15% SDS-polyacrylamide slab gel electrophoresis according to Cleveland et al. (JBC, 1977) yielded minor phosphopeptides of M_r=52,500, 31,000, 24,000, 16,500 and 12,000 and a single major 39,000-dalton phosphopeptide. The peptide map of the 56,000-dalton phosphoprotein was compared to the peptide map of a commercial preparation of RII (phosphorylated in vitro in our assay system) generated by limited tryptic proteolysis. Points of overlap were observed for the 56,000-dalton protein, the 52,500 and 16,500-dalton phosphopeptides and the major 39,000-dalton phosphopeptide which has been identified as the autophosphorylated RII peptide (Takio et al., FEBS Lett, 1980). Also, the denervation-induced change in the cytosolic protein phosphorylation (171 ± 13.4%) can be accounted for by the increased phosphorylation of the 39,000-dalton peptide (157.1 ± 7.3%). These results suggest that some non-impulse mediated neural influence is exerted through the autophosphorylation of RII. Supported by the V.A. Medical Research Service and BRSG funds from Loyola Univ.

273.11 DENERVATION INDUCED DEGENERATION OF NEUROMUSCULAR JUNCTION: THE ROLE OF NICOTINIC INTERACTIONS. S. Rochel-Landau and N. Robbins. Dept. of Anatomy, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106.

The objective of this study is to evaluate the neurotrophic role of nicotinic acetylcholine (ACh)-transmission in the development of denervation dependent fall of muscle resting membrane potential (RMP). If spontaneous (quantal or non quantal) or, action potential evoked ACh release by the motor nerve were the major or only requirement for RMP maintenance, then the denervation induced fall in RMP should be accelerated by nicotinic blockers, which would supply an "instant denervation". Therefore, rat hemidiaphragms with (+N) or without (-N) a 2 cm nerve stump were incubated for different periods of time in organ culture in the presence or absence of nicotinic blockers α bungarotoxin (αBTX, 5 μg/ml) or d-tubocurare (3 μg/ml). Upon completion of the incubation period RMPs and miniature endplate potentials (MEPPs) were measured.

Spontaneous miniature endplate potentials disappeared 12 hrs after nerve section in -N diaphragm strips, and after 18 hrs in +N strips. A 10 mV fall of RMP was attained after 24 hrs in -N strips and after 28 hrs in +N strips, in agreement with in vivo studies (Card, Exp. Neurol. 54, 251, 1977).

Nicotinic transmission was blocked from time zero in αBTX or curare treated muscle, but there was no advance in the time of RMP fall in -N muscles in vitro. Moreover, the nerve stump effect of delaying the RMP depolarization persisted in the continuous presence of αBTX or curare.

Thus, with respect to neuronal regulation of RMP, neither the effects of denervation nor of the long nerve stump are entirely explicable by a nicotinic mechanism. Corresponding in vivo experiments are now in progress. This work was supported by NS 18694 and an NIH Training Grant NS 07118-04.

274.1 DUAL ARBORIZATION OF SINGLE PARASYMPATHETIC AXONS IN THE SPHINCTER AND DILATOR MUSCLES OF THE MOUSE IRIS, Patrick C. Jackson.† Dept. Physiology & Biophysics, Washington University, St. Louis, MO 63110

The intrinsic muscles of the mammalian eye have been found to contain axonal profiles typical of autonomic cholinergic terminals (Nishida & Sears 1969). These are generally accepted as arising from parasympathetic ciliary ganglion neurons. Given that the sphincter and dilator of the iris have opposing actions, one of the possibilities suggested by these observations is that there are groups of neurons within the ciliary ganglion that project separately to these regions. Previous studies of the innervation of the iris have treated the whole population of ciliary ganglion neurons together and do not address this issue. Thus the purpose of the present study was to examine the projection of electrophysiologically and morphologically identified neurons to their target(s) using the intracellular marker horse-radish peroxidase (HRP). The adult mouse was chosen as the preparation for this work.

The eye was dissected from the orbit in continuity with the ciliary ganglion and a length of the occulomotor nerve. In most cases, a single ganglion neuron was impaled with a microelectrode containing HRP. The degree of convergence onto the impaled cell was estimated by recording EPSPs evoked by graded stimulation of the pre-ganglionic axons. Of 101 neurons impaled, 37 were sufficiently well filled to reveal that these neurons exhibit a range in dendritic complexity and that the most complex also received the largest number of inputs. (see also Purves & Hume 1981). The axons of thirteen single neurons were stained and followed to the irides of thirteen preparations. Of these, seven produced fine collateral branches exhibiting varicosities. Six axons arborized and produced terminal varicosities in both the sphincter and the dilator; one produced an arbor only within a small region of the ciliary processes near the root of the iris. There was no obvious relationship between the degree of convergence onto a ciliary ganglion neuron and the size or location of its terminal arbor in the iris.

Injection of HRP into single ganglion neurons with depolarizing current often produced an observable response in the iris; this was always an asymmetric constriction of the pupil. A simple speculation as to the physiological significance of dual distribution of a single axon to the antagonist sphincter dilator muscles is that impulse activity gives rise to a contraction of one and a relaxation of the other. Nishida, S. and Sears, M. (1969) Exptl. Eye Res. 8:292-296. Purves, D. and Hume, R.I. (1981) J. Neurosci. 1:441-452.

†Multiple Sclerosis (Canada) Postdoctoral Fellow.
Supported by MDA and NIH grants to Dale Purves.

274.2 CHANGES IN THE INNERVATION OF CARDIAC GANGLION CELLS ACCOMPANYING NEURONAL GROWTH IN POST-METAMORPHIC XENOPUS LAEVIS. Peter B. Sargent. Department of Structural Biology, Stanford University School of Medicine, Stanford, CA 94305.

Cardiac ganglion cells in Xenopus laevis are innervated by axons which terminate upon both the ganglion cell body and its axon in a number of swellings, called synaptic boutons. Previous work in mature frogs has shown that the number of boutons on the ganglion cell body is directly correlated with its surface area (J. Physiol. 342, in press). Thus bouton density is similar for large and small cells in mature frogs. The experiments reported here were designed to address the question of whether bouton density remains constant during the increase in ganglion cell size that accompanies post-metamorphic growth.

Synaptic boutons terminating on ganglion cell bodies were stained at several stages following metamorphosis with zinc iodide and osmium (ZIO), which by electron microscopy was shown to stain all synaptic boutons. The ZIO procedure also impregnates preganglionic axons and thus permits visualization of the entire presynaptic arborization. Ganglion cells taken from frogs completing metamorphosis have an average diameter of about 15 μm and an average of approximately 4 synaptic boutons on their somata. Both ganglion cell size and bouton number increase continuously throughout post-metamorphic life. The increase in bouton number is accompanied by a marked elongation of the preganglionic axon(s) in contact with the ganglion cell body and by the presence of ZIO-stained growth cones on the cell body. Ganglion cell bodies in frogs nearing sexual maturity have several times the surface area and several times the number of boutons as cells from animals just completing metamorphosis. By contrast, the density of boutons, expressed per unit of somatic surface area, increases only slightly during this same interval. Thus most, but not all, of the increase in bouton number that takes place following metamorphosis occurs in parallel with an increase in cell body size. Although the mechanism generating this "compensatory" growth of both cell size and bouton number is unknown, its effect might be to maintain adequate safety factor for synaptic transmission during the neuronal growth that accompanies post-metamorphic life.

Supported by the National Science Foundation, The March of Dimes Birth Defects Foundation and The Dysautonomia Foundation.

274.3 **TRANS-SYNAPTIC REGULATION OF THE DEVELOPMENT OF CATECHOLAMINE CONTENT IN THE RAT SUPERIOR CERVICAL SYMPATHETIC GANGLION.** A.J. Smolen, T. Lindley*, and L.L. Wright. Dept. of Anatomy, Med. Coll. of Pa./E.P.P.I. Division, Philadelphia, PA. 19129.

It has been shown (Black et al., '72, J. Neurochem., 19:1367) that denervation of the superior cervical ganglion (SCG) of the mouse prevents the normal developmental increase in the activity of tyrosine hydroxylase, the rate limiting enzyme in the synthesis of norepinephrine (NE). A similar finding has been reported in the rat (Thoenen et al., '72, Brain Res., 44: 593), although the time course was not defined. The present experiment was designed to determine if the development of catecholamine content is regulated trans-synaptically by examining SCGs of rats after neonatal ganglionic denervation when reinnervation was either permitted or prevented.

There was no effect on total protein content of the SCG following any of the surgical procedures. In unoperated or sham operated control animals, NE content rose rapidly and reached adult values by one month after birth. In denervated ganglia, NE content failed to increase for the first two weeks, but then attained about 70% of control by one month. There was no significant difference in NE levels between ganglia which were reinnervated and those which were not.

Dopamine (DA) content underwent a normal rise during the first two postnatal weeks, and then fell to reach adult values by one month. In denervated ganglia, the early rise in DA content was abolished, and in the adult, there was no significant difference from controls. By contrast, epinephrine (E) underwent a normal rise during the first postnatal week, and then fell to an undetectable level by one month. In denervated ganglia, there was a temporary enhancement of E content (2-5 times control) at two weeks, but this was followed by a return to control by one month.

In the absence of preganglionic innervation, the content of NE reached 70% of control, while the contents of DA and E were not significantly different than controls. Thus, most of the postnatal increase in NE content in the rat SCG is not mediated trans-synaptically. However, NE content did not completely reach control, even in animals in which reinnervation began after a two week delay. Furthermore, there were temporary effects of denervation on both DA and E contents. Therefore, we conclude that trans-synaptic signals modulate development during the first two postnatal weeks, but that these signals are not essential for the attainment of adult (or nearly adult) levels of catecholamines in the SCG neurons.

(Sponsored in part by NIH Grant NS15952)

274.4 THE EFFECTS OF NEONATAL STRESS ON THE DEVELOPMENT AND INNERVATION OF THE RAT ADRENAL MEDULLA. L. L. Ross and A. Pylpiw*. Dept. Anatomy, The Med. Coll. Pennsylvania, Philadelphia, PA 19129.

The first 3 weeks of life is a critical period in the development of the rat adrenomedullary system. The adrenomedullary cells are proliferating and differentiating rapidly and there is a reorganization of their preganglionic innervation. These maturational events are regulated by central neural mechanisms as well as peripheral endocrine influences. Because the role of the neonatal adrenomedullary system in the response to stress is uncertain, the present experiments were undertaken.

From days 2 to 5, rat pups were subjected to immobilization stress daily for one hour. The animals were allowed to survive until 40 days of age. Controls consisted of littermates who either were isolated from the mother for the same periods without immobilization or were left undisturbed. All 3 groups were distributed according to sex and mother. At sacrifice, adrenal catecholamine levels were determined by HPLC with electrochemical detection. In addition, we assayed for one of the central neural influences regulating adrenomedullary maturation, spinal cord serotonin (5-HT) and its receptors. The former was determined by HPLC/EC and the latter by 5-HT binding.

Both the norepinephrine (NE) and the epinephrine (E) content of the adrenals of neonatally immobilization-stressed females were significantly lower than controls at 40 days of age. Stressed males showed no differences. Although 5-HT levels in the spinal cords of stressed animals were no different than controls, 5-HT receptors in the stressed-females were 20% lower than that of controls. Males showed no differences from controls in 5-HT receptor levels.

These data indicate that neonatal stress causes long-term changes in the catecholamine content of adrenal medullary cells as well as in spinal cord 5-HT receptors and, for the period administered, these stress effects are obtained only in the female.

Supported by the Office of Mental Health of the Commonwealth of Pennsylvania.

274.5 MORPHOGENESIS OF CARDIAC GANGLION NEURONS. R. David Heathcote and Peter B. Sargent. Dept. of Structural Biology, Stanford University School of Medicine, Stanford, CA 94305.

The cardiac ganglion is a derivative of the embryonic neural crest. It is located in the atrium of the heart and is composed of parasympathetic neurons which receive synaptic input from the vagus nerve. In the frog, Xenopus laevis, cardiac ganglion cells undergo their final cell division as early as two days after fertilization and start differentiating soon thereafter. Ganglion cells continue to arise and differentiate well into larval life (Heathcote, Soc. Neurosci. Abstr. 8:258).

The morphogenesis of individual cardiac ganglion cells was examined following intrasomatic iontophoresis of horseradish peroxidase. Mature ganglion cells in adult hearts lack dendrites and have a single axon, which remains unbranched for a considerable distance from the cell body. While some neurons in Xenopus larvae have a similar morphology, many are multipolar and branch profusely. Their processes extend for long distances and can occupy the entire atrium. In addition, many of these larval neurons have fine, unbranched filopodia that emanate both from the cell body and along the length of its axons. These filopodia are as long as 20 microns in larval neurons but are not seen in mature neurons.

These results suggest that during differentiation, supernumerary processes sprout from the cell bodies and axons of immature cardiac ganglion cells which are subsequently "pruned". This transition eliminates primary processes (those that arise from the cell body) as well as secondary and tertiary branches of the axon. It also effectively reduces the percentage of the atrium innervated by any particular ganglion cell. Thus a striking change in morphology is one component of ganglion cell differentiation.

Supported by the American Heart Association, California Affiliate, the National Science Foundation and the March of Dimes Birth Defects Foundation.

274.6 SOMATOSTATIN IMMUNOREACTIVITY IN EMBRYONIC SYMPATHETIC GANGLIA P.H. Chenard* and G.D. Maxwell (SPON: Y. Grimm-Jørgensen) Dept. of Anatomy, Univ. of Connecticut Health Center, Farmington, CT 06032

Some adult peripheral sympathetic neurons contain both catecholamines and somatostatin-like immunoreactivity (SLI) (Hokfelt et al., 1977 PNAS 74: 3587-3591). We have investigated the occurrence of SLI in developing embryonic avian sympathetic ganglia.

SLI was identified by immunohistochemical methods in paraformaldehyde fixed cryostat sections from the trunk region of quail embryos. At stage 18 (Zacchei 1961 Arch. Ital. Anat. Embr. 66: 36-62), which corresponds to embryonic day (E) 4, SLI was detected in cells of the primary sympathetic chain. These chains are the sites of initial aggregation of neural crest cells to form the sympathetic ganglia. The SLI in these cells was seen in the cytoplasm and is absent from the nucleus. At stage 23 (E7), when the lumbosacral paravertebral sympathetic ganglia have reached their definitive location some cells contained SLI and they were often located in the ventral portion of the ganglia. When the antiserum directed against somatostatin was pre-incubated with somatostatin 1-14 immunoreactivity was abolished.

At stage 23-24 lumbosacral paravertebral sympathetic ganglia can be dissected from the embryo and the SLI content determined by radioimmunoassay. These ganglia contained substantial SLI. When calculated on a per mg protein basis, the SLI content declined by 90% between stage 23-24 (E7-8) and stage 26-27 (E9-10) and remained at this low level until at least stage 31-32 (E13-14). When calculated on a per ganglion basis, SLI content declined 75% from stage 23-24 to stage 26-27, indicating that the reduction in SLI content is due to an absolute decrease per ganglion and not solely to growth of tissue that does not contain SLI. In embryonic chick lumbosacral paravertebral sympathetic ganglia, many developmental changes (such as the initiation of synapse formation within the ganglia) occur during the developmental stages equivalent to those at which we find that SLI content decreases in embryonic quail lumbosacral paravertebral sympathetic ganglia. Thus, changes in the expression of SLI may be linked to other key developmental events occurring during sympathetic ganglion maturation.

(Supported by Basil O'Connor Starter Grant 5-289 from the March of Dimes Birth Defects Foundation and NS16115 and a Research Career Development Award (GDM) from the NIH).

274.7 NEURAL INFLUENCES ON MUSCARINIC ACETYLCHOLINE RECEPTORS IN DEVELOPING CHICK HEART. Margaret L. Kirby and Robert S. Aronstam. Departments of Anatomy and Pharmacology, Medical College of Georgia, Augusta, GA 30912.

The development of muscarinic acetylcholine receptors in chick heart was studied from incubation days 5 through 20 using [^{3}H]3-quinuclidinyl benzilate as a probe. The influences of atropine-exposure and removal of postganglionic neurons on receptor numbers and binding properties were investigated.

There was a parallel increase in receptor density in atrium and ventricle until the last half of incubation, when the atrial, but not the ventricular, receptor density increased. This increase was blocked by exposure to atropine on incubation days 11 through 14, but not on days 16 through 19. This specific regional increase was coincident with the appearance of functional cholinergic innervation of the heart. During this same period there was an alteration in muscarinic receptor binding properties in both atrium and ventricle that was characterized by an increase in the proportion of receptors displaying high affinity agonist binding. This increase was blocked in the atrium, but not the ventricle, by atropine exposure on incubation days 11 through 14. Thus, there is a critical period in the development of atrial muscarinic receptors during which the receptors are susceptible to modulation by exposure to an antagonist.

Parasympathetic postganglionic neurons were removed by cauterization of the neural crest over occipital somites 1-3 at embryonic stage 9-10. (This area has been shown to seed the cardiac ganglia.) The number of ganglion cells in the bulbar region of the heart was determined histologically and correlated with the number of muscarinic receptors in the atrium and ventricle on incubation day 15. There was a 66% decrease in the number of bulbar ganglion cells and a 43% decrease in atrial receptor density (on a mg protein content basis). Ventricular receptor density was unchanged. Conotrunical abornormalities result from removal of neural crest over somite S1-3. Since this area provides both structural and neural components to the developing heart, it is possible that children with conotrunical malformations due to neural crest abnormalities may have altered responsiveness to cholinergic agents.

These results indicate a role for cholinergic innervation in the ultimate functional configuration of the cardiac muscarinic system.

(Supported in part by PHS grants HD-17063 and NS-17429).

274.8 PERTURBATION IN ZINC METABOLISM AFTER POSTNATAL LEAD EXPOSURE IN RATS. S. M. Sato*, J. M. Frazier* and A. M. Goldberg. The Johns Hopkins Univ., Dept. of Env. Health Sci., Baltimore, Md. 21205

Previous morphological studies showed that postnatal lead exposure can lead to disturbances in the development of the mossy fiber boutons in the hippocampus. We hypothesized that lead may exert its toxic effect by interfering with zinc found in this neuronal pathway. To test this hypothesis, rat pups were exposed indirectly to lead by adminstering 0.2% lead acetate to dams via the drinking water during lactation for 21 days, while control litters were maintained on tap water. In order to evaluate whether the effects of postnatal lead exposure were selective for hippocampal zinc pools, the hippocampus was compared with the cerebellum in rats 30 and 90 days of age. There were no significant differences noted in total zinc levels in either the hippocampus or the cerebellum after postnatal lead exposure. Furthermore, no differences in the subcellular distribution of zinc were observed between control and lead-treated animals. Since the effects on zinc levels may be more subtle, the amounts of cytosolic zinc-binding species, isolated using Ultrogel AcA 34 gel permeation chromatography, were measured in control and lead-treated animals. In a previous study, we established that cytosolic zinc in the hippocampus was bound to three zinc-binding species. In this study, we determined whether postnatal lead exposure would specifically alter any of these cytosolic zinc pools. A striking decrease was observed in the amount of zinc associated with one of the cytosolic zinc-binding species, a putative zinc-glutathione complex (1.04 $\pm$ .27 ug zinc/g tissue in controls v.s. .61 $\pm$ .13 ug zinc/g tissue in lead-treated animals, $p < .01$). This effect was observed only in the hippocampus of 30 day old lead-treated rats. By 90 days of age, the effect was no longer present. The specific zinc pool affected constituted only 10 to 15% of the total zinc found in the hippocampus. Therefore, by virtue of its small contribution to total hippocampal zinc, a large decrease in this specific pool resulted in undetectable changes in both total and cytosolic zinc levels. These data suggest that lead preferentially affects a zinc pool found in the hippocampus and supports our hypothesis that postnatal lead exposure results in an alteration in zinc metabolism.

Supported by NIH grants, ES07067 and NIEHS ES07094.

274.9 CATECHOLAMINERGIC PROPERTIES OF NEURONS AND PREGANGLIONIC AXONS IN THE RAT CILIARY GANGLION. Story C. Landis, Patrick C. Jackson and John R. Fredieu*. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Neurons in the ciliary ganglion provide cholinergic innervation to the intrinsic muscles of the eye and are in turn innervated by cholinergic preganglionic neurons. In the irides of rats sympathectomized at birth with multiple injections of 6-hydroxydopamine (6-OHDA), we observed an extensive plexus of fibers that were weakly immunoreactive for tyrosine hydroxylase. Since ciliary neurons represent one possible source of these fibers, we examined the ciliary ganglion of normal rats and of rats treated as neonates with 6-OHDA for certain catecholaminergic properties: tyrosine hydroxylase- and dopamine-B-hydroxylase-like immunoreactivity (TH-IR and DBH-IR) and catecholamine (CA) histofluorescence.

TH-IR was present in 10-50% ciliary neurons in normal adult rats. After acute surgical superior cervical ganglionectomy, TH-IR could be detected not only in ciliary ganglion perikarya but also in restricted axonal arbors in the iris. Ciliary neurons did not exhibit detectable DBH-IR or CA fluorescence. Parasympathetic neurons in the sphenopalatine, submandibular and cardiac ganglia of normal rats were not TH-IR. In animals that had been treated as neonates with 6-OHDA, there was an increase both in the number of neurons in the ciliary ganglion that were TH-IR (up to 75%) and in the intensity of the immunofluorescence in many cells.

In addition to the presence of TH-IR in the ciliary neuron perikarya, many cell bodies were surrounded by TH-IR fibers. The fibers were present in normal rats, in rats surgically sympathectomized and examined 4 days later and in rats chemically sympathectomized at birth and examined as adults. Ultrastructural examination of TH immunoperoxidase material disclosed labelling of many preganglionic synapses. The synaptic terminals in the ganglion possessed obvious CA fluorescence, but no detectable DBH-IR or small granular vesicles after permanganate fixation. The presence of prominent pericellular CA fluorescence made it difficult to determine unequivocally whether ciliary neuron perikarya possessed any CA fluorscence.

Our studies to date indicate that some ciliary neurons in normal adult rats have TH-IR while other parasympathetic neurons do not. Further, they suggest that the level of TH-IR can be influenced by sympathectomy. In addition, many preganglionic axons contain TH-IR and catecholamine, probably dopamine. Studies are in progress to examine further the developmental and functional significance of the presence of catecholaminergic properties in these parasympathetic neurons and their preganglionic input. Supported by NINCDS and the American Heart Association.

274.10 ^{3}H-NOREPINEPHRINE UPTAKE AND CATECHOLAMINE CONCENTRATIONS IN DEVELOPING CHICK HEART FOLLOWING 6-HYDROXYDOPAMINE TREATMENT. D.E. Stewart and M.L. Kirby. Department of Anatomy, Medical College of Georgia, Augusta, GA 30912.

Developing sympathetic nerves first reach the heart of the embryonic chick on incubation days (ID) 10-11. Sympathetic cardiac innervation is not complete until after hatching (ID 21). Previous work has revealed that 6-hydroxydopamine (6-OHDA) treatment is effective in reducing neuronal ^{3}H-norepinephrine (^{3}H-NE) uptake in chick atria as early as ID 12. Furthermore, ingrowth of new adrenergic fibers occurs rapidly (between 24-72 hours following 6-OHDA). Daily treatment with 6-OHDA during the latter part of embryonic development is necessary to produce a maximal lesion. To achieve a maximal lesion using 6-OHDA to destroy cardiac sympathetic terminals during development, we administered 6-OHDA (100 mg/kg daily, saline with 0.5 mg ascorbate/ml as vehicle) in ovo by injecting through the airspace onto the inner shell membrane on ID 13-19. ^{3}H-NE uptake assays on chick atria (in vitro) were performed on ID 20. Specific neuronal uptake of ^{3}H-NE was defined as total uptake minus uptake in the presence of desmethylimipramine, a specific uptake blocker. Treatment with 6-OHDA produced a 67% decrease in specific neuronal ^{3}H-NE uptake from saline-injected controls. To correlate cardiac catecholamine concentrations with ^{3}H-NE uptake data, the concentrations (pg/mg tissue) of norepinephrine (NE) and DOPA in whole hearts were determined by high performance liquid chromatography. 6-OHDA treatment produced a statistically significant (53%) decrease in NE concentration in whole hearts compared with saline-treated controls (90 $\pm$ 10 pg/mg versus 190 $\pm$ 10 pg/mg, mean $\pm$ SEM). These same hearts had a 33% increase (not significant) in DOPA concentration (40 $\pm$ 10 pg/mg versus 30 $\pm$ 10 pg/mg). This data indicates that a significant proportion of catecholamines remains in the heart despite 6-OHDA treatment. The source of these catecholamines is thought to be a combination of (1) the few sympathetic nerves remaining in the heart, (2) circulating catecholamines from the adrenal medulla and (3) possibly de novo synthesis within the heart. The inability to completely eliminate ^{3}H-NE uptake in 6-OHDA treated hearts may indicate a population of 6-OHDA-resistant sympathetic nerves or ^{3}H-NE uptake by desmethylimipramine-insensitive cells.
Supported by NIH Grant HD 17063.

274.11 FORMATION OF PARAVERTEBRAL SYMPATHETIC CHAIN GANGLIA IN CHICK EMBRYOS. J.W. Yip. Dept. of Physiol., Sch. of Med., Univ. of Pittsburgh, Pittsburgh, PA 15261.

The formation of the paravertebral sympathetic chain ganglia was investigated using quail-chick chimera. Single segments of quail neural tube with overlying neural crest at stages 11-15 (40-55 hrs) were transplanted homotopically onto chick embryos at similar stages. Transplantations were made at both cervical and thoracic levels. Eighteen embryos were sacrificed at stages 33-38 (7 1/2-12 days), serially sectioned and examined for the presence of quail cells in the paravertebral sympathetic chain ganglia.

Neural crest cells originating from a single segment of transplanted quail neural tube populated several contiguous sympathetic ganglia. The extent of neural crest migration was similar whether the transplantation was performed at stage 11 or 15. Thus, at least within those stages, there does not appear to be any temporal factor which determines which rostrocaudal ganglia are populated by a given segment of the neural crest. There was also no difference in the extent of neural crest migration whether embryos were sacrificed at stage 33 or stage 38. Sympathoblast migration thus appears to be completed by stage 33.

In cervical and thoracic levels, sympathetic ganglia arise largely from neural crest cells of the corresponding levels. Neural crest cells at a particular segment, however, will migrate longitudinally along the neural axis. Neural crest cells originating from a single segmental level migrate on average 2 segments rostrally and 3 segments caudally. Individual sympathetic ganglia of the cervical and thoracic levels may therefore contain neural crest cells that originate from 6 different segments of the neural axis. Whereas the majority of neurons within a particular ganglion arise from the same segment of the neural crest, there are diminishing contributions from progressively more distant segmental levels.

Selective synaptic connections based on the positional attributes of pre- and postsynaptic elements has been suggested in the mammalian sympathetic system and in the innervation of transplanted intercostal muscles by axons of the cervical sympathetic trunk (Wigston, D.J. & Sanes, J.R., Nature, 299:464, 1982). The segmental origin of sympathetic ganglion cells in the neural crest may be a basis for their innervation by preganglionic axons arising from particular levels of the spinal cord.

Supported by BNS-8210028 and MH 30915.

274.12 CONTRIBUTION OF SYMPATHETIC CARDIAC INNERVATION AND ADRENAL CATECHOLAMINES TO BASAL HEART RATE IN NEONATAL RATS. D.C. Tucker. Preventive Med., Washington Univ. Sch. of Med., St. Louis, MO 63110

Controversy exists about maturation of autonomic controls over the heart in neonatal rodents. Because enhanced sympathetic influence on heart rate was identified in neonatal rats genetically predisposed to hypertension (i.e. SHR rats, Tucker & Johnson in press), tonic sympathetic and parasympathetic controls of the heart during the neonatal period were further investigated. Using a series of selective pharmacological treatments, the present study suggested tonic cardiac control by both sympathetic cardiac nerves and circulating adrenal catecholamines by two days of age.

Rat pups from 26 Sprague Dawley (SD) and 9 Charles River (CR) litters were implanted with subcutaneous silver wire electrodes the day prior to testing. Recordings of ECG were made from freely moving pups at either 2, 5 or 8 days of age. After obtaining a stable baseline heart rate, bretylium tosylate (25 mg/kg) was injected to block release of NE from sympathetic cardiac nerves; pups were then returned to the dam. At 2.5 hours post bretylium injection, heart rate was sampled and the muscarinic receptor blocker atropine methylnitrate (10 µg/kg) was injected. Ten minutes later, the ganglionic blocker hexamethonium bromide (30 mg/kg) was administered to block release of adrenal catecholamines. Finally, the cardioselective β-adrenergic receptor blocker atenolol (1 mg/kg) was injected to directly antagonize chronotropic stimulation of the heart. All pups received this sequence of subcutaneous drug injections.

Heart rate responses were similar in CR and SD pups ($p>.10$). As expected, both basal heart rate and heart rate after autonomic blockade increased with age. Profile analysis indicated a significant contribution of sympathetic cardiac neural stimulation to basal heart rate (i.e. -48 bpm post bretylium treatment; $F(1,25=22.2$ $p<.0001$); the bradycardia was similar at 2, 5 and 8 days. Adrenergic influences of adrenal origin also contributed significantly to basal heart rate at all ages ($F(1,24)=130$, $p<.0001$); the heart rate decrease after hexamethonium injection was greater at 5 days than at 2 days of age (i.e. -56 bpm vs -27 bpm; $F(2,24)=3.41$, $p<.05$). Subsequent direct blockade of β-adrenergic receptors with atenolol further reduced heart rate at all ages ($F(2,24)-3.41$, $p<.0001$). Parasympathetic control was not evident at any age tested ($p>.50$).

These data suggest substantial tonic adrenergic influence on basal heart rate in newborn rats which comprises control by both sympathetic cardiac nerves and adrenal catecholamines.
(Grant #5 T32 HL07456)

274.13 REORGANIZATION OF SYMPATHETIC EFFERENT AND SACRAL AFFERENT INPUTS TO PARASYMPATHETIC GANGLIA OF THE URINARY BLADDER IN RESPONSE TO PARTIAL DENERVATION PRODUCED BY INTERRUPTION OF THE SACRAL PREGANGLIONIC OUTFLOW. W.C.de Groat and M.Kawatani*, Dept. of Pharmacology, Univ.of Pittsburgh, Pittsburgh, PA 15261

The urinary bladder (UB) receives an excitatory input from sacral parasympathetic (PSYM) preganglionic axons in the pelvic nerve (PN) and a mixed input from sympathetic (SYMP) efferent pathways in the hypogastric nerve (HGN) including: (1) inhibition of the detrusor muscle via β-adrenoreceptors, (2) inhibition of transmission in bladder ganglia (BG) via α-adrenoreceptors and (3) excitation of the trigone. Both nerves also contain afferent pathways from the UB. Clinical studies have suggested that injury to the PSYM innervation can lead to the unmasking of excitatory SYMP inputs to the UB which contribute to the development of autonomous bladder activity. This possibility was explored in chloralose anesthetized cats subjected to unilateral transection of sacral ventral roots 2-36 weeks before the experiment.

In these cats stimulation (stim) of the normal PN, but not the denervated PN elicited bladder contractions (BC). Repetitive stimulation (10-30 Hz) of the HGN ipsilateral to the denervation (DEN) produced a bimodel BC consisting of a large early contraction (EC) and a small late contraction (LC). HGN-stim on the normal side produced only the EC. The LC, detected 4-30 weeks after denervation, was blocked by atropine (10-20 μg/kg i.a.) but was not changed by α-adrenergic blocking agents (phenoxybenzamine, 0.5 mg/kg or dihydroergotamine, DHE, 0.1-0.5 mg/kg). The EC was not affected by these drugs. HGN-stim on the DEN-side also elicited a discharge in ipsilateral bladder postganglionic nerves (B-PGN) (latencies of 20-30 msec and at thresholds of 1-1.5V). The discharge was blocked by hexamethonium (C_6, 1-10 mg/kg) indicating it was synaptically mediated. HGN-stim at high intensities (7-10 V) on the normal side elicited long latency (80-200 msec) C_6 resistant, axonal volleys in ipsilateral B-PGN. Spontaneous B-PGN firing occurred on the DEN-side only. Stim of DEN-PN (10-20 Hz, 15-20 V) elicited a prolonged (3-5 min) increase in the firing. HGN-stim (10-30 Hz) on the DEN-side depressed the firing. Administration of DHE blocked the HGN inhibition and unmasked an excitatory response. The excitatory effects produced by stimulation of the PN or HGN were not blocked by atropine or C_6. Substance P enhanced and GABA, norepinephrine and leucine-enkephalin inhibited the spontaneous firing.

In summary, interruption of the sacral preganglionic outflow to the UB led to the reorganization of synaptic connections in BG which was reflected in: (1) the appearance of spontaneous firing (2) the formation of sympathetic cholinergic excitatory inputs and (3) the formation of non-cholinergic, non-adrenergic excitatory inputs from afferent axons remaining in the pelvic nerve.

274.14 EXCITATORY EFFECT OF SUBSTANCE P (SP) ON PARASYMPATHETIC GANGLIA IN THE CAT URINARY BLADDER, A.M.Booth, M.Kawatani*, T. Whitney* and W.C.de Groat, Dept. of Pharmacology, Sch. of Med., Univ. of Pittsburgh, Pittsburgh, PA 15261

The urinary bladder receives an afferent and preganglionic efferent innervation from the sacral cord via the pelvic nerve. Several weeks after surgical interruption of the preganglionic outflow in the cat, electrical stimulation of the de-efferented pelvic nerve elicits a prolonged discharge in the bladder postganglionic fibers which is resistant to cholinergic blocking agents. One explanation for these observations is that afferent pathways in the pelvic nerve can excite denervated ganglion cells in the bladder. Since some pelvic afferents contain SP,the present experiments were undertaken to determine whether exogeneous SP would mimick the non-cholinergic excitatory ganglionic response.

In normal ganglia in situ, intraarterial injection of SP elicited bladder contractions (1-10 μg/kg), facilitated postganglionic potentials evoked by pelvic nerve stimulation (0.1-1 μg/kg) and elicited an asynchronous postganglionic discharge (1-10 μg/kg. The SP induced bladder contractions were blocked by the SP-antagonist, D-Pro^2, D-Phe^7, D-Trp^9 -SP (1-5 μg/kg), whereas the ganglionic excitatory effects of SP were blocked by D-Arg^1, D-Pro^2, D-$Trp^{7,9}$, D-Leu^{11}-SP (5-10 μg/kg). The SP antagonists did not alter the ganglionic responses to tetramethylammonium or acetylcholine. SP discharges were not blocked by hexamethonium, atropine, leucine-enkephalin (L-ENK) or norepinephrine (NE) but were blocked by GABA. In denervated ganglia which exhibited spontaneous firing, SP elicited a discharge in lower doses (0.001-0.1 μg/kg). L-ENK, NE and GABA blocked the spontaneous firing but only GABA blocked the responses to SP.

Normal bladder ganglia in vitro, perfused with Krebs-Ringer solution responded to exogenous SP (30-100 μg) injected into the 10 ml tissue bath. With intracellular recording responsive cells were depolarized in a dose dependent fashion 1 to 2 minutes following administration of the drug. The depolarization lasted 2-3 minutes. Microliter quantities of 100 μg/ml SP pressure ejected onto the ganglia produced similar effects. The amplitude of epsp's or intracellular depolarizing current injections necessary to fire the cells was lowered and some cells were sufficiently depolarized to fire repetitively in the absence of any apparent synaptic or intracellular stimulation.

In conclusion, exogenous SP produces non-cholinergic excitation in the normal and parasympathetically denervated bladder ganglia. The effect of SP is mediated by depolarization of the ganglion cells. Further experiments will be required to determine whether SP is the mediator of spontaneous and pelvic nerve evoked non-cholinergic firing in denervated ganglia.

DEVELOPMENTAL DISORDERS

275.1 COBALT FOCAL EPILEPSY IN INFANT RATS. G. T. Golden, R. G. Fariello, P. F. Reyes*. Dept. of Neurology and Research Jefferson Medical College, Philadelphia, PA 19107 and VA Medical Center, Coatesville, PA 19320.

The occurrence of severe epileptic seizures early in life can lead to neuronal loss and impairment of brain development. In this study we have attempted to produce a chronic model of focal epilepsy in infant rats. Subdural placement of a cobalt powder-agar plug was performed on infant Long Evans hooded rats at six days of age. The cobalt powder was mixed with agar and saline and the resulting plug (1.5 mm) was placed overlying sensorimotor or parital cortex. Regular electroencephalographic recordings through scalp needles were conducted on a weekly basis until the rats reached adulthood. At the first EEG recording session (14 days of age) electrographic abnormalities were observed. Electrographic activity was characterized by a paucity of interictal spikes. Eighty percent of the animals presented ictal discharges, in the form of burst of polyspikes or spike and wave complexes, not dissimilar in their morphology from the discharges that have been observed in naive rats and interpreted by some investigators as normal EEG variants of sleep activity. Ictal discharges were longer lasting and appeared more frequently in cobalt treated rats. Aside from facial myoclonus, no behavioral convulsive seizures were observed. In general the neonatal brain of the rat seems to be more refractory to the epileptogenic action of cobalt than the adult brain. Histopathological features of the cobalt lesions will be described and compared to lesions seen in adult rats.

275.2 PRENATAL METHYLAZOXYMETHANOL(MAM) TREATMENT PRODUCES SPONTANEOUS HYPERACTIVITY IN NEONATAL AND ADULT RATS. P.R. Sanberg, T.H. Moran*, K.L. Kubos*, P.G. Antuono* and J.T. Coyle. Depts. of Neuroscience and Psychiatry, Div. of Child Psychiatry, Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205 and Dept. of Psychology, Ohio Univ., Athens, Ohio.

Administration of the antimitotic agent MAM to rats on day 15 of pregnancy produces a marked hypoplasia of the telencephalon in the offspring with a resulting hyperinnervation of ascending neurotransmitter systems to the forebrain (Johnston and Coyle, J. Neurochem. 34:1429,1980). The present study examined the pattern of spontaneous locomotion in these MAM lesioned rats.

Pregnant rats were injected i.p. with 20 mg/kg of MAM on day 15 of gestation; control rats were injected with saline. The offspring of both groups were subsequently placed at various ages into computerized Animal Activity Monitors (Omnitech Electronics) and tested over a 24-hour period. These monitors provided detailed information of the animals' pattern of locomotion using 21 different variables measuring activity in the horizontal and vertical planes. A repeated measures ANOVA was used to analyze the statistical significance of the data.

The results demonstrated that, compared to controls, the MAM-lesioned rats exibited significant increases in nocturnal locomotor behaviors beginning at 10 days old and continuing into adulthood. The pattern of this nocturnal hyperactivity in adults was demonstrated by significant increases in total distance traveled, excursions around the walls of the apparatus, stereotypic and vertical movements. The amount of time moving in the horizontal plane, however, did not differ between groups, reflecting that a significantly greater average speed was exhibited by the MAM lesioned animals.

These findings suggest that rats with forebrain microencephaly induced by prenatal treatment of MAM may provide a useful model for hyperkinesis in humans which is also correlated with a significantly higher rate of cerebral atrophy (Nostralla et al, Soc. Bio. Psychiat. Abstr.,42,1983). Furthermore, it is likely that the hyperinnervation of catecholaminergic terminals within the cortex of MAM lesioned rats may mediate this spontaneous hyperactivity. Supported by MH26654, the Pratt Family and Friends HD grant, and the Tourette Syndrome Association.

275.3 DEFICIENCY OF D-AMINOACID OXIDASE (DAAO) IN THE REELER MOUSE. J. Vamecq , A. Goffinet & F. Van Hoof (SPON: M. David-Remacle) Université catholique de Louvain and I.C.P., UCL 75.39, B-1200 Brussels Belgium.

The function and cellular localization of DAAO remains largely controversial. In mice, the only organs where this enzyme displays a significant activity are the cerebellum, brain stem, jejunum and kidney cortex. In the latter organ it has been located in the peroxisomes. In the cerebellum, DAAO is associated with sedimentable particles which lack the other peroxisomal oxidases (Gaunt, G.L. & de Duve, C., J. Neurochem., 26:749, 1976). In the cerebellum of 20 homozygote reeler mice (agranular mutants), DAAO activity assayed with 0.1 M D-proline represented 40.5 ± 6.7% (standard deviation) of matched control mice, and in the kidney, 54 ± 8. There was no overlapping value between normal and reeler mice. A similar deficiency of DAAO was found with D-alanine and D-valine as substrates. Control enzymes (ß-hexosaminidase, sulfatase C and cytochrome c oxidase) were normal in both organs from reeler mice. In the kidney and in the liver, catalase activity was also normal.

The probability that cerebellar DAAO belongs to the granule cells is stressed by experiments with methylazoxymethanol according to J. Slevin et al. (Dev. Neurosci. 5:3, 1982). When this alkylating agent is injected intraperitoneally to normal new-born mice it prevents multiplication of granule cells and affects drastically the appearance of DAAO activity (50-fold less than controls on day 21). Kidney DAAO and control enzymes in both organs are unaffected by this treatment.

In the mutant, deficiency of cerebellar DAAO probably reflects the scarcity of granule cells but this provides no explanation for the lesser activity of DAAO in kidney. The biochemical observations could be reconciled if two or more isoenzymes contributed to the DAAO activity; the reeler mutant could then be characterized by the absence of the major cerebellar isoenzyme.

This work was supported by NIH Grant 9235, the Belgián F.R.S.M. and F.N.R.S, Crédit au Chercheur 1.5.598.83F. J. Vamecq is ICP-fellow.

275.4 EARLIER IS WORSE: BEHAVIORAL, ANATOMICAL AND ELECTROPHYSIOLOGICAL DIFFERENCES BETWEEN EARLY AND LATE POSTERIOR PARIETAL INJURY IN RATS. B. Kolb, C. Holmes* and I.Q. Whishaw. Dept. of Psychology, University of Lethbridge, Lethbridge, Alberta,Canada, T1K 3M4

Rats with removal of the posterior parietal cortex (area 7) in adulthood were compared behaviorally, anatomically, and electrophysiologically to rats with similar removals at 1 or 5 days of age. Ablations in adulthood impaired foot placing on a narrow beam and retarded the learning of the position of a hidden target in the Morris water task. Use of the forepaws during feeding, puzzle latch opening, grooming, tongue use, food hoarding, and learning of a radial arm maze was normal. In contrast, infant operates were impaired at nearly every test administered. There was no evidence of any sparing of function and the youngest operates were worst of all on many tests.

Examination of cerebral weight, neocortical thickness, cross-sectional cortical area, barrel field and thalamic organization showed that although the neonatal operates had much smaller areas of cavity, they had significantly smaller brains, the posterior cortex was abnormally thin, the barrel fields were abnormally organized and the thalami were shrunken and abnormally formed. The extent of the abnormalities were far worse in the 1 day than in the 5 day operates, although both groups differed from the adults. Electrophysiological studies showed that cortical and hippocampal atropine-resistant and atropine-sensitive EEG in freely moving animals was normal in all groups in spite of the anatomical abnormalities. The results of stimulation of the motor cortex in acute preparation shall also be reported.

The results suggest that early brain damage may have greater effects upon the animal than similar injury in adulthood. Early injury appears to prevent the development of some behavioral capacities that an equally extensive injury at maturity would not have destroyed, behavioral effects that are correlated with abnormal cortical and subcortical morphogenesis. These results provide the evidence in support of Hebb's prediction in 1949 that early brain damage should have far more deleterious effects upon brain and behavior than similar injury at maturity.

275.5 THE EFFECTS OF PERINATAL CHLORPROMAZINE ADMINISTRATION ON CEREBELLAR MONOAMINES IN THE ADULT RAT. R.S. Hannah*, D. Parkinson , A.W. Spira and S.H. Roth. Depts. of Anatomy and Pharmacology and Therapeutics, University of Calgary, Calgary, Alberta, Canada. T2N 4N1.

Previous studies have established that perinatal chlorpromazine (CPZ) administration produces both a reduction in Purkinje cell number and chronic alterations in Purkinje cell dendritic architecture. Since the monoamine system in the cerebellum has been suggested to possess some inductive properties during development, an attempt was made to examine its status in perinately CPZ treated, mature animals. CPZ (15 mg/Kg) was administered intramuscularly to timed pregnant Long-Evans rats beginning on day 18 post coitus. Maternal CPZ or normal saline administration was continued on a once daily basis until day 21 postnatal.

Animals were allowed to eat and drink ad libitum. At birth, the litter size was culled to eight pups to negate any intra-litter nutritional variation based on unequal litter size. The animals were sacrificed at six months of age, and the cerebella removed. Two histochemical methods were utilized to examine the monoamine fiber distribution; these were the fluorescence histochemical formaldehyde method of Falck and Hillarp (J. Histochem. Cytochem. 10: 348-354, 1962) and the glyoxylic acid fluorescence histochemical method of Lindvall and Bjorklund (Histochem. 39: 97-127, 1974). High pressure liquid chromatography with electrochemical detection (HPLC) was used to measure cerebellar levels of noradrenaline, dopamine and serotonin.

Both histochemical fluorescence methods demonstrated the following alterations in the treated animals: a paucity of fluorescent axon terminals in the molecular layer, a concentration of fluorescent axon terminals in juxtaposition to the basal region of the Purkinje cell body and numerous fluorescent axon terminals in the granule cell layer. Monoamine levels as measured with HPLC demonstrated a statistically significant reduction in noradrenaline, dopamine and serotonin in the treated animals. The most severe reduction, approximately 35%, occurred with serotonin, with noradrenaline and dopamine reduced by approximately 20%. These results suggest that perinatal CPZ administration produces chronic alterations in the cerebellar monoamine system.

(Supported by MRC, Canada, and Alberta Mental Health).

275.6 ECTOPIC NEURONS IN THE BRAIN OF THE AUTOIMMUNE MOUSE: A NEUROPATHOLOGICAL MODEL OF DYSLEXIA? G.F. Sherman, A.M. Galaburda, and N. Geschwind. Department of Neurology, Beth Israel Hospital and Harvard Medical School, Boston, MA.

The brain in developmental dyslexia has been found to be structurally abnormal (Drake, J. Learn. Dis., 1:9, 1968); Galaburda and Kemper, Ann. Neurol., 6:94, 1979). Micropolygyria, abnormal cytoarchitecture (especially in the language regions), and ectopic neurons in cortical layer I were seen in the left hemisphere. Recently, Geschwind and Behan (PNAS, 79:5097, 1982) have found a relationship among developmental learning disabilities (including dyslexia), lefthandedness, and autoimmune disease. Since autoimmunity and dyslexia cluster in lefthanded individuals and their families, it was decided to examine an experimental animal that develops spontaneous autoimmune disease for the presence of brain abnormalities that might be similar to those seen in the human dyslexic brain.

We examined the brains of the New Zealand Black (NZB) mouse and its F1 hybrid (NZB/W). Six 30-day old NZB, six NZB/W, and six C57Bl/6J control brains were examined. An additional six NZB/W brains were examined at 90 days of age. The groups included both males and females. The animals were perfused with 10% formalin, the brains were removed, and after fixation were embedded in cellodin. They were then coronally sectioned at 30 micrometers and every fifth section was stained with cresylechtviolett for Nissl substance.

Ectopic collections of neurons were present in layer I of the cerebral cortex in four of the 30 day-old New Zealand mouse brains. The 90 day-old and control brains showed no abnormalities. Three of the abnormal brains had unilateral ectopic cells and one had a bilateral accumulation which was more severe on one side. There were also subtle distortions in the underlying cortical architecture. The abnormal brains were distributed equally over sex and three of the four brains were from the NZB strain. The two largest collections of ectopic neurons were present in the right hemisphere at the border between the primary and secondary somatosensory cortices.

We hypothesize that an immune-mediated process may be the cause of the abnormal neuronal migration that results in heterotopias in both the human dyslexic and NZB mouse. The NZB mouse provides an experimental model in which to explore this question. (Supported by NIH grants 14018 and 07211).

275.7 HYPERACTIVITY RESULTING FROM NEONATAL INFECTION WITH A HOST RANGE MUTANT HERPES SIMPLEX TYPE 1 VIRUS. L. S. Crnic, L. J. Yamamoto,* and L. I. Pizer*. Departments of Pediatrics, Psychiatry and Microbiology, University of Colorado School of Medicine, Denver, CO 80262.

Balb/c mice were injected subcutaneously with 10^6 plaque forming units of a host range mutant herpes simplex type 1 virus in the first day of life. The mutant had been selected to grow on primate but not mouse cells in vitro and so produced a mild infection in the neonatal mice. Control groups were either injected with the vehicle (media and cell fragments) or not manipulated. Mice were sacrificed at 3, 7, 14, 21, 28 and 60 days for determination of brain virus titers. At the latter 3 time points, open field activity was measured in an automated open field before sacrifice. Mice allowed to grow to adulthood were tested on step down passive avoidance learning and spatial discrimination learning in a radial arm maze.

Virus was evident in the brains by 3 days, reached peak titers of 2.9×10^3 plaque forming units per brain by 21 days, and viable virus was not detectable at 60 days in most brains. Small brain and body weight deficits were apparent throughout development in the virus-treated mice but were gone by adulthood. Hyperactivity in the virus-treated mice consisted of high activity levels throughout development and a failure to decline to adult levels by 60 days of age, even though no virus was detectable in most brains at that age. At 60 days of age, the infected mice crossed an average of 144 $\pm$ 34 squares while the vehicle group crossed 76 $\pm$ 26 and the controls 67 $\pm$ 37 squares in the open field. Prior studies have shown the hyperactivity to persist into adulthood in the absence of infectious virus. The adult mice also showed deficits in passive avoidance learning (7.25 $\pm$ 2.49 trials to criterion for infected versus 4 $\pm$ 0.35 for control), although their performance on a radial arm maze was not impaired, indicating that the passive avoidance learning deficit was probably not due to a learning deficit but rather difficulty in inhibiting behavior.

This sort of virus infection is a potential cause of the specific behavioral disorders of childhood. In addition, the selective destruction of brain areas by mild virus infections could be a useful tool for the study of brain function.

275.8 DEVELOPMENTAL DYSLEXIA: THIRD CONSECUTIVE CASE WITH CORTICAL ANOMALIES. A.M. Galaburda, G.F. Sherman and N. Geschwind. Department of Neurology, Beth Israel Hospital and Harvard Medical School.

Developmental dyslexia is a now widely recognized learning disability in which there is primarily a difficulty in the acquisition of reading and writing. Three consecutively-studied brains of dyslexics have shown cortical anomalies. The first, reported by Drake (J. Learn. Dis., 1:9, 1968), showed abnormal gyral folding and excessive numbers of ectopic white matter neurons. These were found in the parietal lobes bilaterally. Portions of the corpus callosum were thought to be thin. There was an associated cerebellar angioma that had bled. The second case, published by Galaburda and Kemper (Ann. Neurol., 6:94, 1979) exhibited cortical and subcortical heterotopias, micropolygyria, and abnormal cortical lamination in the left hemisphere. These anomalies were found in the cingulate gyrus and perisylvian cortex, and the micropolygyria occupied most of the left planum temporale. We report here the third case, that of a 14 year-old dyslexic boy with a positive family history of dyslexia. The brain weighed 1597 gm (large) after formalin fixation. It was embedded whole in cellodin and cut in gapless serial histological sections. Every 20th section was stained for Nissl and the adjacent section for myelin. The sylvian fissures and planum temporale were approximately symmetrical. There were a multitude of focal accumulations of ectopic neurons in layer I of the cortex of the left cerebral hemisphere only. They involved all lobes, but predominantly perisylvian regions. No micropolygyria was seen. The cortical architecture in neighboring areas showed laminar disorganization and primitive features. These consisted of poor delineation of laminae, relative curtailment of granular layer IV, paucity of neurons in layer III and large pyramids in layers V and VI showing random orientation.

Geschwind and Behan (PNAS, 79:5097, 1982) have found a relationship among developmental learning disabilities (including dyslexia), lefthandedness, and autoimmune disease. The NZB and NZB/W immune mice have shown mainly unilateral clusters of ectopic cells in layer I similar to those found in the two dyslexic brains studied by us. We postulate that the cortical abnormalities seen in the dyslexic brains and NZB mice may result from an alteration of the immune process during the late stages of neuronal migration.

(Supported by NIH grants 14018 and 07211)

275.9 PHYSIOLOGICAL AND BEHAVIORAL EFFECTS OF PREGESTATIONAL ADMINISTRATION OF MORPHINE TO MALE RATS Sarah C. Lantz* and Robert A. Jensen. Developmental Biopsychology Laboratory, Southern Illinois University, Carbondale, Illinois 62901.

In recent years an extensive body of information has been gathered on the consequences of female exposure to drugs and other agents both prior to and during gestation. Some literature also suggests similar consequences following male exposure to various agents. However, little systematic investigation of this finding has been carried out. Some chemical agents may affect aspects of male reproductive function and also the development of offspring.

Friedler (Fed. Proc., 1974, 33, 515) reported that morphine administered to male mice prior to mating produced offspring with lower birth weights than controls. In that study, male animals were withdrawn from the drug for five days prior to mating and females were drug free throughout the procedure. The present research was designed to determine whether pregestational administration of morphine to male animals produces teratogenic effects. In this research we investigated both physiological and behavioral parameters.

Twenty-seven male Long Evans rats (13 morphine, 14 saline) between 90 and 150 days of age were injected (s.c.) on a five-day schedule. On Day 1, each animal received a 10.0 mg/kg injection of morphine sulphate or saline control. On Days 2 through 5, each animal received both a morning and an evening injection with an increase of 2.5 mg/kg per injection. Following the last injection males were withdrawn for 5 days. They were then placed in mating cages with estrus females for 5 days. At birth, pups were culled to 6-8 per litter. Animals were weighed on Days 1, 4, 6, 8, 12, 16, 20, and post-weaning. Free-fall righting was tested on Day 1, negative geotaxis on Day 4, inhibitory avoidance on Days 24 and 25, active avoidance on Days 26 and 27, mother-pup interaction by a pup retrieval task and nest building. No significant differences in free-fall righting, visual placing, or in the mother-pup interactions were observed. Morphine-sired pups performed significantly poorer on the negative geotaxis test, an indication of delayed nervous system development. On Day 15, mophine-sired pups were significantly more active. Additionally, these animals performed significantly better in retention of an inhibitory avoidance task and in acquisition of an active avoidance task. In summary, lower weights, deficits in the negative geotaxis reflex, altered activity levels, and altered performance in two learning tasks indicate that pregestational administration of morphine to males may affect their offspring.

275.10 UNILATERAL CEREBRAL ANOXIC/ISCHEMIC DAMAGE IN NEWBORN RATS INCREASES HYPERTHERMIA-INDUCED SEIZURE SENSITIVITY. J. Olson and D. Holtzman. Dept. of Psych. and Neurol., Tulane University School of Medicine, New Orleans, LA 70112.

Unilateral ligation of the carotid artery combined with exposure to a hypoxic environment produces neuronal necrosis and gliosis ipsilateral to the ligation in adult (Levine S, Am J Pathol 36:1, 1960) and in seven-day-old rats (Rice et al., Ann Neurol 9:131, 1981). We have extended this model of hypoxic/ischemic brain damage to two-day-old animals in order to study the mechanism(s) responsible for the increased frequency of febrile seizures which often accompany the pathologic sequelae of neonatal cerebral ischemia.

Two day old Sprague-Dawley rat pups were anesthetized with Ketamine and immobilized on a 37°C heating pad. A partial thymectomy was performed followed by exposure of the common carotid artery between the sternohyoid and sternocleidomastoid muscles. A #8-0 silk suture was passed under the artery and tied tightly. Following recovery from anesthesia, the animals were placed in 100% humidified N_2 at 37°C for up to 40 min. The animals were allowed to recover before returning to the dam. Some pups were given sham operations or were exposed to anoxia without carotid ligation.

All of the ligated animals exposed to 40 min of anoxia died within 24 hrs while less that 25% of the animals exposed to 30 min of anoxia died during this period. No animals died following anoxia exposures of 15 min or less. At 5 and 10 days of age a seizure was induced by hyperthermia in ligated-anoxia-exposed animals and control litter mates. At 5 days of age the seizure temperature threshold (STT) was not significantly different in experimental compared to control animals (mean $\pm$ SEM = 42.8 $\pm$ 0.2°C for experimental animals, 42.8 $\pm$ 0.1°C for controls). At 10 days of age the STT was significantly lower for animals exposed to 30 min of anoxia after carotid ligtion (mean $\pm$ SEM = 43.5 $\pm$ 0.2°C for experimental animals, 44.5 $\pm$ 0.1°C for controls, $p<0.001$). The STTs of sham-operated animals and of animals exposed to anoxia alone were not different from controls.

The data indicate that brain damage produced by anoxic/ischemic exposure in neonatal rat pups produces increased sensitivity to hyperthermia-induced seizures in later life. This may provide a relevant model of febrile seizures in brain-damaged children.

This research was supported by grants to D. Holtzman from the NIH (NS16256) and the Cerebral Palsy Foundation.

275.11 BEHAVIORAL THERMOREGULATION IN THE DEVELOPING TWITCHER MOUSE. Charles E. Olmstead, UCLA, School of Medicine, Mental Retardation Research Center Group at Lanterman State Hosp., Pomona, CA 91769

The mutant twitcher mouse (B6.CE/twi) which was first described by Duchen et al. (Brain 106, 1980) is an enzymatically authentic (Kobayashi, et al. Biochem. Med. 27, 1982) model of the recessively transmitted Globoid cell (Krabbe's) leukodystrophy, a demyelinating disease of infantile onset. Clinical records of patients often show exaggerated swings in body temperature in the middle and late stages of this severe deymyelinating disease. Although we have no reason to suspect that a thermoregulatory defect plays a role in the pathogenesis of globoid cell leukodystrophy we were interested in determining the extent of the regulatory problem and whether it might be apparent prior to the other manifestations of the disease. The specific enzyme deficit in both the human and the twitcher mouse is galactosylceramidase and affected, carrier and normal individuals can be determined by enzyme assays done on clipped tail. The studies reported here were carried out to 1) develop a better understanding of the developmental pathophysiology of this demyelinating disease and 2) to develop non-invasive methods for evaluating treatment regimens as they are developed.

Thermal gradient. The thermal gradient, modeled after Ogilvie and Stinson (Canad. J. Zool. 44, 1966) and Satinoff et al. (Science 193, 1976), consisted of a 120 x 15cm aluminum plate with one end coupled tightly to a laboratory hotplate and the other immersed in ice. The gradient remained stable at between 15 and 40°C for up to 8 hours with only occassional replenishment of ice. Over the ages from 2 to 20 days of age mice in groups of 3 to 5 were placed randomly on either the warm or cold end of the gradient and their movements and postures noted for 3 hours. Usually the final position of individual mice on the gradient was stable for the last hour of observation.

Enzyme assays. Following the final trial, 0.5cm lengths of tail were clipped and galactosylceramidase was determined by an assay adapted from Suzuki (Meth. Enzymol. 50, 1978). Affected homozygous animals were readily identified prior to the onset of physical symptoms.

Mice from litters containing only heterozygotes and homozygously normal animals huddled together at progressively cooler portions of the gradient as a function of age. Mice from litters containing homozygously affected animals showed a much wider dispersion over the entire gradient and those with the lowest enzyme levels, who subsequently developed the disease, were concentrated at the colder extremes of the gradient. Although there was considerable between litter variability, at risk mice were easily distinguished from heterozygotes and normals within the litter as early as eight days of age on the basis of position. These data suggest that there may be an early thermoregulatory deficit in the twitcher mouse.

275.12 LOCOMOTOR DEVELOPMENT IN THE TWITCHER MOUSE. Richard Sugerman and Charles E. Olmstead. UCLA, School of Medicine, Mental Retardation Research Center Group at Lanterman State Hosp., Pomona, CA 91766.

The mutant twitcher mouse (B6.CE/twi) which was first described by Duchen et al. (Brain 106, 1980) is an enzymatically authentic (Kobayashi, et al. (Biochem. Med. 27, 1982) model of the recessively transmitted Globoid cell (Krabbe's) leukodystrophy. The specific enzyme deficit is galactosylceramidase, and affected and carrier individuals can be determined by enzyme assay done on clipped tail. The studies on the development of locomotor behavior reported here were carried out to 1) develop a better understanding of the functional pathophysiology of this demyelinating disease and 2) to develop non-invasive methods for evaluating treatment regimens.

Enzyme assays. At between 10 and 15 days of age, 0.5cm lengths of tail were clipped and galactosylceramidase was determined by an assay adapted from Suzuki (Methods Enzymology 50, 1978). Affected homozygous animals were readily identified prior to the onset of physical symptoms.

Gait development. Beginning at 12 days of age mice were placed twice daily onto a well-inked and brightly lit stamp pad and encouraged to run across an 8.5 x 11 inch sheet of paper to a darkened receptacle. The resultant footprints were analyzed on a digitizing tablet for step length and width and angles between fore and hind paws (Schallert et al. Science 199, 1978; Hruska et al. Life Sci. 25, 1979). All mice showed an increase in running speed and length and width of step across 15 to 30 days of age. Affected mice were not distinguishable from normals, on the basis of gait, until after 25 days of age at which time a broadening of the distance between the rear foot prints is seen. This initial symptom was followed by a blurring of discrete digit prints by 30 days of age through limb dragging to a cessation of locomotion by 35-40 days of age. In all cases the hind limbs were affected prior to the front. All animals died in the 7th or 8th week of life.

Open field. In a 50 x 25cm open field normal mice showed characteristic changes in behavior as a function of age and experience. Affected twitcher mice showed normal patterns of locomotion, exploration, rearing and grooming into the 4th week of life. As the locomotor changes occurred parallel changes were seen in the open field behavior, with rearing, exploration and locomotion disappearing in that order. Area grooming, which in normal mice developed in a rostral-caudal direction from face to body to tail, was arrested at the body stage in the 5th week and reduced to vestigal facial swipes by 35-40 days of age.

These data demonstrate that despite the enzyme defect the twitcher mouse shows normal functional development well into the 3rd and 4th weeks of life.

275.13 A BIOCHEMICAL STUDY OF THE EFFECT OF HYPOXIA ON CEREBELLAR DEVELOPMENT. M.C. Yu* (SPON: J. McArdle). Dept. of Anatomy, New Jersey Medical School, Newark, New Jersey 07103.

A study was made to determine the effect of hypoxia on the biochemical maturation of the rat cerebellum. Five-day old rats (Sprague-Dawley strain) were culled into 8 pups per dam, and placed into an hypoxic chamber where they were exposed for 7 consecutive days to a low oxygen environment of 10% O_2 and 90% N_2. During the period of hypoxia, the pups were taken out of the chamber twice daily at 9:00 AM and 4:00 PM for supplemental feedings with an enriched liquid diet in order to circumvent malnutritional factors due to decreased milk production by the dams. For control, another group of rats, also culled to 8 pups per litter, were exposed to ambient air only and nursed by their dams. On postnatal day 12, one week after the hypoxic exposure, all the rats were taken out of the chamber. One group of rats was killed immediately while the other groups were cross-fostered by other, normal lactating dams, and killed at postnatal days 17, 22, 40 and 90, respectively. At day 12, the cerebellar weight of the hypoxic rats was 45% of the control, and was 68, 77, 86 and 85% of the control group, at 17, 22, 40 and 90 days. The total contents of DNA, RNA, protein and cerebroside of the hypoxic rats at day 12 were 54, 50, 42 and 35% of the control, respectively. These values, with the exception of protein, lagged significantly behind the control throughout the experimental periods, so that at day 90, the contents of DNA, RNA, and cerebroside of the hypoxic rats were 83, 75 and 72% of the control. The protein contents of the hypoxic rats reached to 62, 90, 92 and 108% of the control at 17, 22, 40 and 90 days, respectively. These data suggested that hypoxia, imposed during the period of rapid neurogenesis (production of microneurons), caused a permanent cell deficit in the cerebellum as indicated by the reduction in DNA content. The process of myelination also appeared to be interfered with.

(Supported by NIH Grant 1 RO1HD12089)

275.14 AGE AND MUTATION DEPENDENT CHANGES IN MYELIN MBPs. S. Greenfield#, E. L. Hogan, G. Gantt#, M. J. Weise#, S. W. Brostoff#. Dept. of Neurology, Med. Univ. So. Carolina, Charleston, SC 29425.

The immunoblot technique was used to identify myelin basic proteins (MBPs) in myelin and homogenates of sciatic nerve and brain from young and adult normal and mutant (qk, jp, msd) mice. Proteins were detected with anti-MBP serum following electrophoretic transfer from slab gels (modified from the system of Swank and Munkres). Using this gel system which resolves the 18.5K MBP into 18K and 18.5K components, we were able to detect differences in the basic protein composition of adult CNS and PNS myelin. A protein doublet was seen in the 21.5K region of PNS myelin which appeared as a single band in CNS myelin. An 18K basic protein was present in CNS myelin, which was absent in PNS myelin. The 17K basic protein in PNS myelin can be resolved into two distinct protein bands. Two basic protein bands migrating between 18.5K and 21.5K were seen in CNS homogenates of young qk mice but not in normal mice. The PNS basic protein compositions of qk, jp, and msd mice appeared qualitatively but not quantitatively similar to normal control mice. During development, the 18K CNS MBP appeared prominently during the active phase of myelinogenesis. At 10 and 15 days of age the 14K MBP in brain homogenates contains both 14.4K and 14.8K components while at later ages only the 14.4K MBP persists. Supported in part by NIH grants NS 12044 and NS 11867.

276.1 DEVELOPMENT OF GABAergic NEURONS IN PRIMARY CULTURES OF RAT RETINA: COMPARISON OF NEURONAL AND MIXED CULTURES. I. Pech* and P.V. Sarthy (Spon: T. Kennedy). Depts. of Physiol. & Biophys, & Ophthalmology, University of Washington, Seattle WA 98195.

In a previous report, we described conditions which allowed for survival of primary cultures of dissociated embryonic rat retinal neurons for 1 mo in culture in the virtual absence of glial (flat) cells [Soc. Neurosci. Abst. 8:49.4, 1982]. These cultures developed the ability to synthesize GABA from glutamate, to release GABA in a K^+-stimulated, Ca^{2+}-dependent manner, and to take up GABA via both high and low affinity uptake systems.

We now report that, by growing the cells in bicarbonate-containing medium in a 5% CO_2 atmosphere, one can obtain cultures containing both retinal neuronal and glial cells (mixed cultures). Under these conditions, the initial morphology is quite different than in the neuronal cultures. In the latter, the cells grow individually and begin to show some extension of neurites as early as 1 d.i.v. In the mixed cultures, the cells exist in undifferentiated clusters for the first week in culture. Towards the end of this time, one can begin to distinguish the flat cells from the neurons and, with increasing time thereafter, the cells acquire their mature appearance. The flat cells spread out as thin sheets on the surface of the dish, and the neurons grow on top of them. Although many more neurons attach and grow in the mixed cultures than in the neuronal cultures, the total survival time is similar in both conditions. Few neurons survive beyond 1 mo in culture, but the flat cells can be maintained for 2 mo or more.

The parameters of GABA function which were examined in the neuronal cultures were also examined in the mixed cultures. During the first week, when the cells existed in undifferentiated clusters, GABA synthesis, GAD activity and K^+-stimulated, Ca^{2+}-dependent GABA release remained low. Thereafter, they rose rapidly to reach a peak after 2 wk. GABA uptake, as measured biochemically, began to rise after 4 d.i.v. and peaked at 12 d.i.v. As in the neuronal cultures, uptake was seen to be energy-, temperature- and Na^+-dependent and to be blocked by 1 mM GABA, DABA and nipecotic acid. 1 mM β-alanine blocked uptake to a far greater extent in the mixed than in the neuronal cultures. Kinetic studies demonstrated the presence of both high and low affinity uptake systems for GABA uptake. With light microscopic autoradiography, little GABA uptake could be distinguished in the cell clusters which existed during the first week in culture. Thereafter, GABA uptake occurred in an increasing number of neurons with time. Virtually all neurons were labeled by 3 wk in culture. The glial cells demonstrated only low levels of labeling at all times in culture.

276.2 DIFFERENTIAL COMPATABILITY OF HYPER- AND HYPO-DOPAMINERGIC STATES WITH INFANTILE AND ADULT LOCOMOTOR PATTERNS OF MICE. M. J. Forster * and Z. M. Nagy. Bowling Green St. Univ., Bowling Green, OH 43403.

When removed from their home cage and tested in an open-field, infant mice aged 1-10 days typically pivot by turning with the forelimbs whereas older mice walk with both fore- and hindlimbs. While this age-related behavioral change is thought to reflect maturation of underlying neural substrates, few studies have attempted to identify those specifically involved. The present studies addressed the possible role of the dopamine (DA) neurons, as histochemical and biochemical studies suggest that DA systems undergo considerable postnatal development, and because the DA system appears to be involved in locomotor behavior of adults.

In initial studies, separate groups of mice aged 9, 11, or 13 days were injected with the DA antagonist, haloperidol (.2mg/kg), the agonist, apomorphine (.25mg/kg), or the appropriate vehicle and tested for open-field activity for 5 min, 0-, 30-, or 60-min postinjection. Pivoting and walking were measured in terms of the number of initiations, cumulative duration, and average duration per initiation. Control groups showed age-related change in locomotor patterns, with pivoting dominant at 9 days of age and walking dominant at 13 days. Comparisons among the age groups indicated that haloperidol completely attenuated the emergence of walking between 9 and 13 days whereas pivoting was not equivalently affected. Conversely, apomorphine promoted walking and greatly attenuated pivoting as early as 9 days of age.

In a second study, .1mg/kg haloperidol effectively reduced walking of 11- and 13-day-olds without affecting pivoting of 9-day-olds, while neither .2 nor .1mg/kg affected pivoting of 5-day-olds. Apomorphine (.25mg/kg) reduced pivoting of 5-day-olds but elicited behavior best described as crawling rather than walking.

The differential effects of hypo- and hyper-dopaminergic states upon walking and pivoting suggest that development of walking behavior may involve the DA system, while pivoting behavior may involve other neurotransmitter systems.

276.3 EFFECTS OF PRENATAL MATERNAL ADMINISTRATION OF CHLORPROMAZINE ON RAT OFFSPRING MOTOR FUNCTION AND MOTIVATED BEHAVIOR. J. Graefe* and M. Caplan. Dept. of Psychology, Adelph University, Garden City, NY 11530.

The use during pregnancy of psychopharmacological agents affecting the central nervous system (CNS) has markedly increased in recent years. Experimental studies indicate prenatal administration of CNS drugs to rats can lead to functional and behavioral changes in the offspring (Golub and Kornetsky, 1978; Coyle, Wayner and Singer, 1976). The present study examined behavioral changes of offspring of dams treated with chlorpromazine (CPZ) during gestation. CPZ was chosen because of its use by pregnant women and probable influence on developing dopaminergic (DA) systems in the brain resulting in possible alterations of motor function and motivated behavior.

Timed pregnant Sprague-Dawley rats were obtained from a breeder. Single daily injections of saline (SAL) or 4 mg/kg CPZ were administered s.c. on days 7-13 of gestation. At birth, litters were culled to 8 and pups were fostered to lactating dams until 21 days of age. Beginning on day 22, all offspring were tested for 3 consecutive days every 10 days on a different behavioral test each day (i.e. day 22- open-field for 5 mins., day 23- inverted screen, day 24- runway test for food). Subjects were food deprived 24 hrs. prior to the runway test. This sequence was repeated 4 times.

Both SAL and CPZ offspring showed increasing activity scores in the open-field over the 4 test occasions. The CPZ group consistently showed lower activity scores than did the SAL group except on day 22 when both groups were the same. Results of the runway test showed that the CPZ group had shorter latencies leaving the start box and faster run times (RTs) to the goal box than did the SAL group. Sex differences were found only in the runway test: females in both groups had shorter latencies and faster RTs than did males in their respective groups. The inverted screen test did not yield differences between treatment groups.

It appears that prenatal CPZ influences the development of central DA systems. Although the mechanisms mediating the effects of prenatal CPZ are largely unknown, the finding that CPZ offspring had lower activity scores in the open-field than did SAL offspring and shorter latencies and faster RTs in the runway suggests that prenatal CPZ treatment modifies the development of central DA systems that mediate locomotor activity and motivated behavior. Tonge (1973) has reported altered monoamine levels in rodents treated prenatally with CPZ. Whether the drug directly affected fetal DA-systems or induced maternal alterations of humoral influences on the fetal DA-CNS is not evident from these data, although CPZ has been shown to cross the placental barrier (Ghetti, Gliozzi and Cassano, 1969).

276.4 UNMASKING OF A NEONATAL SOMATOVESICAL REFLEX IN ADULT CATS BY ACTIVATION OF SEROTONIN AUTORECEPTORS WITH 5-METHOXY-N,N-DIMETHYLTRYPTAMINE (5-MeODMT). K.B.Thor, T.Hisamitsu*, W.C.deGroat, Dept.of Pharmacol.,Sch.of Med.,Univ. of Pittsburgh,Pgh.,PA 15261

In neonatal kittens micturition is induced by the mother cat licking the perigenital (PG) region. At about the time of weaning the perigenital-bladder (PG-B1) excitatory reflex disappears and throughout adult life tactile stimulation (stim.) of the PG region inhibits micturition. Transection of the spinal cord in adult animals causes the return of the PG-B1 excitatory reflex.

The present experiments explored the possibility that the serotonergic (5-HT) system may be involved in the switch from PG stim. induced BL excitation to inhibition during development. This hypothesis seemed reasonable since 1) 5-HT inhibits B1 activity and 2) spinal transection both eliminates 5-HT in the sacral spinal cord and results in the expression of the same reflex patterns seen in kittens where the 5-HT system has not matured. To see if the absence of 5-HT function in kittens and spinal cats can account for the presence of a PG-B1 excitatory reflex, we decided to suppress the 5-HT system in normal adult cats in an attempt to unmask the PG-B1 excitatory reflex. This was done by stimulation of presynaptic 5-HT autoreceptors with low doses of 5MeODMT to decrease 5-HT release. The response of the bladder (B1) to tactile stim. of the PG region and electrical stim. of the pelvic and pudendal (PUD) nerves was monitored by recording B1 pressure changes and B1 efferent neural activity in chloralose anesthetized cats.

During control periods, both large rhythmic B1 contractions and reflexes recorded from B1 postganglionic fibers in response to pelvic nerve stim. were abolished or greatly reduced by PG or PUD stim. Low doses (5-50 µg/kg i.v.) of 5MeODMT suppressed or eliminated the inhibition of the B1 by PG or PUD stim. in 7 of 7 cats and furthermore, in 4 of those 7 cats changed the inhibition to excitation. Rhythmic B1 activity was increased slightly or unchanged. The effect was maximal 3-5 min after injection and lasted 20-25 min. Larger doses (200-500 µg/kg i.v.) of 5MeODMT produced opposite effects: in those cases where a PG-B1 excitatory reflex was unmasked by low doses, larger doses suppressed the reflex by 50% and rhythmic B1 contractions were abolished, possibly via activation of postsynaptic 5-HT receptors.

In summary, 5MeODMT in normal adult cats produced somatovesical reflex patterns similar to those seen in kittens and chronic spinal cats. Reports that low doses of 5MeODMT depress 5-HT neurons suggests that in normal adult cats the 5-HT system suppresses the excitatory PG-B1 reflex and facilitates the inhibitory component of PG stim. Thus, during postnatal development the switch from PG induced B1 excitation to inhibition may reflect maturation of 5-HT bulbospinal control over spinal reflex mechanisms.

276.5 DEVELOPMENTAL CHANGES IN DOLICHOLS OF MAMMALIAN BRAIN. Y. Sakakihara*, W.C. Landesman*, R.I. Goldberg*, J.J. Volpe, Depts. Ped., Neurol., Biol. Chem., Washington Univ. Sch. Med., St. Louis, MO 63110

Dolichols are long-chain alcohols composed of five-carbon isoprene units derived from the cholesterol biosynthetic pathway. These compounds serve as carriers of saccharide moieties in the biosynthesis of glycoproteins. Because of this function and the importance of glycoproteins in differentiating systems, it is likely that dolichols play critical role(s) in one or more aspects of brain development. In this study, we define the content of free and esterified dolichols in developing rat brain.

Animals were studied from late fetal life to 13 postnatal months. Dolichols were isolated from brain by organic extraction, separated from other lipids by column chromatography, and identified and quantitated by HPLC. A striking increase in brain content of dolichols was defined. Thus, levels of total dolichol were extremely low in brain of the 17-day fetus (0.3 µg/g wet weight) and newborn (0.6 µg/g). Postnatally, a gradual increase in levels occurred until 15 days when a rapid rise to a peak level of 9.0 µg/g at 40 days was observed. (Thereafter little change was found until two to three months of age when a second rise in brain dolichol levels occurred, reaching the high levels (18 µg/g) previously reported in aging brain.) Approximately 65-70% of total brain dolichol was in the free form during the time of the early developmental increase.

In contrast to dolichols, cholesterol deposition exhibited a distinctly different developmental pattern. Thus, cholesterol content increased from relatively low levels in neonatal brain to nearly peak levels after approximately 21 days, when the rapid rise in dolichol levels was just beginning.

Coincident with the striking increase in brain dolichols with brain development, a distinct change in the distribution of the several molecular species that comprise the total dolichol fraction was demonstrated. Thus, with development the dolichols of shorter chain length (C80, C85) increased, whereas those of longer chain length (C95, C100) decreased.

The data thus define a striking increase in brain dolichols during the time period in the rat that corresponds to active myelination. The fact that the developmental increase in brain dolichols is independent of the increase in brain cholesterol suggests that the regulation of formation of dolichol is also an independent process. This is of particular interest because the precursor unit of dolichols is derived from the cholesterol biosynthetic pathway. The timing of the developmental increase in brain dolichols suggests that these compounds are involved in biosynthesis of glycoproteins critical for the progress of myelination. (Supported by NIH grant RO1-HD-07464).

276.6 PYRUVATE DEHYDROGENASE ACTIVITY IN REGIONS OF THE RAT BRAIN DURING POSTNATAL DEVELOPMENT. Roger F. Butterworth, Jean-François Giguère*, France Landreville* and Micheline Pelletier*. Lab. of Neurochemistry, Clinical Research Institute of Montreal (University of Montreal), Montreal, Quebec, Canada H2W 1R7.

The limited capacity of neonatal brain to oxidise glucose probably results from a maturational delay in the activity of the pyruvate dehydrogenase complex (PDHC). As part of a study to more fully elucidate the role of PDHC in brain development, the activity of the enzyme complex was measured in 7 brain regions of the male rat at different times during the postnatal period using an arylamine acetyltransferase coupled assay (Ksiezak-Reding et al., J. Neurochem., 38, 1627 (1982). 3 days after birth, PDHC activity was less than 15% of adult levels in all brain regions studied with the exception of hypothalamus and medulla-pons (30% of adult values in both cases). PDHC in these regions increased rapidly during the postnatal period to attain adult levels by 21 days postnatally, some 5-10 days ahead of those found in cerebral cortex, striatum and hippocampus. These differences in PDHC maturation likely reflect the greater degree of early maturity in these phylogenetically older brain structures.

Cerebellar PDHC developed significantly more slowly than in other regions of the brain, at all times after birth, to attain only 40% of adult levels by 21 days. Cerebellum, in contrast to other regions of brain, acquires most of its neuronal complement postnatally and the pattern of maturation of cerebellar PDHC found in the present study is paralleled by:-

(i) Increased incorporation of glucose into cerebral amino acids;

(ii) Development of parallel fibre synaptogenesis;

(iii) Development of specific cerebellar glutamate receptor binding sites.

These findings suggest that PDHC may play an important role in the development of metabolic compartmentation and maturation of cerebral function in the rat.

(This work was supported by a grant from The Medical Research Council of Canada (MA-7620).

276.7 OPIOID-SENSITIVITY OF NEURONS IN ORGANOTYPIC CULTURES OF FETAL MOUSE DORSAL ROOT GANGLION (DRG) EXPLANTS GROWN EITHER ATTACHED TO SPINAL CORD OR IN ISOLATION. A. Chalazonitis and S.M.Crain, Dept of Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y. 10461

Opiate agonists selectively decrease the Ca^{++} component of the action potential (AP) recorded from dissociated chick and mouse embryo DRG somas (Mudge et al, PNAS 76, '79; Werz and Macdonald, Br.Res.239 '82). In contrast, no significant alterations in the APs generated by adult mouse DRG neurons were detected during exposure of freshly isolated ganglia to opioids (Williams & Zieglgänsberger Neurosci.Lett.21, '81). To clarify this difference we compared the opiate sensitivity of sensory neurons in organotypic explants of DRGs attached to spinal cord cross-sections vs isolated DRG explants. Intracellular recordings were made with beveled micropipettes (40-80 MΩ) on DRG neuron somas (20-50 µm diam.) at 3-5 wks. in vitro. Opiate agonists were tested on DRG spikes evoked (at 0.1 Hz) in a balanced salt solution (BSS) containing 5 mM Ca^{++} and 5 mM Ba^{++} in order to enhance inward current through Ca^{++} channels and block delayed voltage-dependent K^{+} channels. A "hump" developed in the repolarizing phase of the spike under these conditions which was blocked by 5-10 mM Co^{++} (9 out of 9 tested neurons), confirming Ca^{++} mediation of this hump. Opiate agonists were bath-perfused (0.5 ml/min) for test periods up to 10 min. In 26 out of 50 DRG neurons (i.e.52%) in 47 DRG-cord explants, the duration of the Ca^{++} component of the AP was reduced in the presence of D-ala-D-leu enkephalin (DADLE) at 10 µM. On the other hand, in 26 isolated DRG explants, 27 out of 33 neurons (i.e.82%) were sensitive to DADLE (χ^2 test of significance: $p<0.005$). The decrease in duration of the Ca^{++} hump ranged from 20-100%, with means of $54\pm4\%$ for neurons in DRG-cord explants and $47 \pm 4\%$ in isolated DRG explants. These effects were reversed after return to Ca^{++}-Ba^{++}BSS and prevented by perfusion of the specific opiate antagonist, naloxone (3 µM), together with DADLE in all 12 neurons pre-tested for sensitivity to DADLE. In about 30% of the opiate-sensitive neurons (in both attached and isolated cultures) the shortening effect on the duration of the Ca^{++} hump "desensitized" within several min of exposure to the agonist; this opioid effect was stably maintained in the rest of the cells in 4-10 min tests. Also, although the duration of the Ca^{++} hump of the somatic DRG AP was significantly shorter in attached vs isolated cultures, some of the opiate-insensitive neurons still generated prominent Ca^{++} humps.

The data obtained thus far are consistent with the hypothesis that as the central terminals of fetal DRG neurites participate in the formation of opioid synaptic networks within the dorsal-horn region of DRG-cord explants (Crain et al, Br.Res. 133 '77, 157 '78) the opioid sensitivity of the perikaryal region of these DRG neurons may decrease.

(Supported by NIDA grant DA-02031 to S.M.C.)

276.8 ONTOGENETIC DEVELOPMENT OF THE SPECIFIC ^{3}H-NITRENDIPINE BINDING SITES IN THE RAT WHOLE BRAIN. H. Matsubayashi*, S. Kito, E. Itoga, M. Togo*, F. Ishizaki* and K. Mizuno*. Third Dept. of Int. Med. Hiroshima Univ. School of Med., Hiroshima, Japan 734.

The characteristics of Ca channel antagonist binding sites in the central nervous system with use of tritiated nitrendipine (^{3}H-NTD) as ligand have been investigated by several authors for the past two years. The authors studied ontogenetic development of ^{3}H-NTD binding sites in the rat brains. Materials were Wistar strain rat whole brains of 1 day, 3 day, 7 day, 14 day, 1 month and 2 month postnatal ages. In addition, brains of the prenatal stage were studied. The tissues were homogenized and washed repeatedly in 50mM Tris-HCl buffer (pH 7.4 at 25°C) and the final pellet was resuspended in Tris-HCl buffer of a tissue concentration of 50 mg original wet weight/ml. The authors performed saturation studies using tissue aliquots of 500ul at 25°C after 60 min incubation with various concentrations of ^{3}H-NTD. Specific binding was defined as the amount of ^{3}H-NTD displaceable by 1 uM nifedipine. Furthermore, autoradiography of ^{3}H-NTD binding sites of these brains of various ontogenetic stages using cryostat sections incubated with 0.6 nM ^{3}H-NTD for 2hrs at 0°C was done. As results, the mean Kd value of fetal, 1-day, 3-day, 7-day and 14-day-old rats was 868.6±236.7(s.d.)pM, whereas that of 1-month and 2-month-old rats was 467.0±25.4pM and binding affinities became higher after 1 month after birth. As for B max, it was 143.0±59.9 fmol/mg protein as the mean value of these various stages and there were no considerable changes in the course of ontogenetic development. Autoradiographic study revealed that ^{3}H-NTD binding sites were diffusely distributed in the gray matter, especially in the hippocampus, interpeduncular nucleus and olfactory bulb. Autoradiographic distribution of these binding sites was also studied ontogenetically.

These days, where ^{3}H-NTD binding sites are located, on the cell membrane or some of the intracellular organellas, has been discussed. It is known that the ionic dependence of the inward current of the action potential is changed from Ca ion to Na ion channels in the fetal development of the amphibian embryo and Ca ion channel is considered to be early developed. In our study, Kd values of Ca antagonist binding sites decreased after birth. This result was noteworthy, since in most of neurotransmitter receptor sites Bmax usually increased, while Kd values remained unchanged. This means that development of ^{3}H-NTD binding sites are not parallel to increase of synaptic sites in the developmental stages.

276.9 DEVELOPMENTAL REGULATION OF CELL SURFACE ANTIGENS IN CHICK NEURAL RETINA. G.J. Cole*, S.A. Dyer*, M.A. Derby* and L. Glaser* (SPON: W. Landau). Department of Biological Chemistry, Washington University School of Medicine, St. Louis, MO 63110

A previous study in our laboratory has described a fluorescent-activated cell sorter (FACS) method that permits the rapid screening of hybridomas that are secreting antibodies which react with surface antigens of retinal cell subpopulations (Dyer et al., Dev. Br. Res., in press). Using this method we have identified a number of monoclonal antibodies that bind distinct populations of embryonic neural retina cells. Two of our monoclonal antibodies react with surface antigens that are developmentally regulated and neural-specific. The monoclonal antibody C1H3 recognizes an antigen present on approximately 70% of the total cell population in dissociated cells from 9 day retina. This antigen is localized to cell bodies of the inner nuclear and ganglion cell layers, and to processes of the plexiform and nerve fiber layers. It has been identified by immunoblotting as a 140 kd polypeptide, which during development is diminished in concentration concomitant with the appearance of a 170 kd component.

A second monoclonal antibody, D1C4 reacts with a minor subpopulation of retina cells (5% of total cells). Immunohistochemical localization of the antigen recognized by D1C4 indicates that it may be present on ganglion cells. This conclusion is based on the presence of the antigen on processes of the inner plexiform and nerve fiber layers. The D1C4 monoclonal antibody recognizes two polypeptides: a major component with a MW of 260 and a minor species of 210 kd. Both antigens decrease as a percentage of total protein during development,and are only slightly detectable by immunoblotting at embryonic day 17.

The restriction of these antigens to distinct subpopulations of retina cells should prove invaluable in the isolation and molecular characterization of defined neural cell types. (Supported by Grants GM18405, GM28002, NSF PCM 80 11973, and 5 P01 GM28002-03.)

276.10 MOLECULAR EVENTS DURING CEREBELLAR DEVELOPMENT
W. Wille, H. Schaal*, and U.A.O. Heinlein*, Institute for Genetics, University of Cologne, Köln, West-Germany

During the histogenesis of the cerebellum (Cb) of wildtype (+/+) and staggerer (sg/sg) mice, regulation of some glycosidases has been demonstrated (Wille & Trenkner, J. Neurochem. 34:443, 1981; Wille et al. J.Neurochem. 40:235, 1983). In order to correlate the enzyme activities with their natural substrates, an attempt was made to describe more generally the alterations of glycolipid and protein patterns during the ontogeny of the Cb.

Gangliosides, which occur in relative high concentrations in the nervous system, are a natural substrate for the particulate neuraminidase, one of the glycosidases mentioned above. The developmental profiles of 6 major gangliosides show individual alterations during histogenesis. The most striking feature is a transient accumulation of GD3 with the highest molar percentage (23%) at P7. The rapid decrease during the second week after birth reduces the relative amount of GD3 to an adult level of 3.2%. Localization studies with the monoclonal Ab R24 (Pukel et al. J.Exp.Med. 155:1133, 1982) demonstrate that at P7 GD3 is only expressed in the external (and partly internal) granule cell layer, the site of postnatal neuron proliferation. In adult Cb only faint background staining is detectable. We interpret the neonatal dominance of GD3 as a consequence of massive de novo synthesis of membrane material rather than as a functional conversion of cell surfaces. The sg/sg Cb exhibits only in adult animals ganglioside differences to the +/+. The increase of GD3 and GM1 may be due to astrogliosis, and the decrease of GD1a and GT1b may be caused by degeneration of neurons.

The Purkinje cell specific protein P400 (Mikoshiba et al. Dev.Neurosci. 2:254, 1979) and the N-CAM protein (Edelman & Chuong, PNAS 79:7036, 1982) have been described during cerebellar development. By aid of one- and two-dimensional SDS-PAGE, sensitive silver staining, and labeling with radioactive sugars, amino acids, phosphate, and sulphate we characterized additional age- and genome-specific proteins: SP47 (IEP 6.4) appears only in the sg/sg Cb with an age-dependent increase corresponding to P400 in +/+; SD70 (IEP 8.4) increasing in postnatal +/+ Cb and not in sg/sg; SD28/SD29 doublet disappears in sg/sg Cb after P30; MP195/MP74 are two nuclear proteins occuring only in Cb older than P7; EP56 (IEP 6.0) increases between E11 and P0 and is absent in adult Cb; GP165, a glycoprotein specific for the molecular layer; EP45 (IEP 4.5), a phosphorylated protein with a kinetic similar to EP56; histones, which differ significantly in the sg/sg Cb. Data from in vitro translation of Cb poly(A)mRNA in reticulocyte lysate and Xenopus oocytes will be presented.

276.11 TECTAL REGULATION OF PROTEIN TRANSPORTS IN REGENERATING GOLDFISH OPTIC FIBERS. Myong G. Yoon+, Larry I. Benowitz++, Ellen R. Lewis++ and Frank A. Baker+. +Dept. of Psychology, Dalhousie University, Halifax, N.S. Canada, B3H 4J1, and ++Dept. of Psychiatry Harvard Medical School, McLean Hospital, Belmont, MA 02178.

Differential changes in the rapidly-transported proteins along the regenerating optic fibers were examined in the presence or absence of their main target, the optic tectum, to test its regulative effects in adult goldfish as follows: Both optic nerves were crushed in all experimental fish at the same time. In one half, the tectum was also ablated bilaterally so that the regenerating optic fibers were prevented from interacting with the tectum; (R-T) group. In the other half of the fish, the optic nerves were allowed to regenerate back to the tectum; (R+T) group. The complements of rapidly-transported proteins in the regenerating optic fibers were compared between these two groups, by differentially labeling with H^3- and C^{14}- proline, and 1 or 2 dimensional gel-electrophoretic separations of retinal proteins. At early post-operative periods (10, 15 and 20 days), no significant difference between the two groups was observed. Beginning on day 24, however, (R+T) group showed a prominent increase in 110-150K dalton species of retinal proteins relative to (R-T) group. Their differential increase in this protein species reached a peak on day 30, and subsided thereafter. In contrast, another species of the retinal proteins with molecular weights in 24-27K dalton range was found to label considerably more in (R-T) group than in (R+T) group, beginning on day 30, and persisting up to 5 months. These results indicate that the optic tectum exerts differential regulative effects on synthesis and/or transport of proteins in regenerating optic fibers; in particular, their interaction with the tectum enhances the 110-150K species, whereas it diminishes the 24-27K component. If the regenerating optic fibers are prevented from interacting with the tectum, the 24-27K component remains enhanced for several months.

(Supported by grants from NSERC and MRC of Canada to MGY and from U.S. NINCDS to LIB).

276.12 FOOD DEPRIVATION INCREASES 5HT AND 5HIAA IN NEONATES BUT NOT OLDER RAT PUPS. F.M. Scalzo and L.P. Spear. Dept. of Psychology, State University of New York at Binghamton, Binghamton, NY 13901.

The serotonergic system has been implicated in the modulation of suckling behavior during development. While serotonergic antagonists markedly reduce suckling behavior in neonatal rat pups (Spear & Ristine, 1982), such antagonists conversely appear to induce suckling behavior in weanling rats (Williams et al., 1979; Ristine & Spear, 1982). The presence or absence of food has been shown to influence serotonin (5HT) synthesis in adult animals, presumably through influencing serum levels of the precursor amine tryptophan (e.g., Fernstrom & Wurtman, 1974) and its binding to serum albumin which is potently inhibited by serum nonesterified fatty acids (NEFA) (Yuwiler, 1977). It has been suggested that food deprivation may increase brain 5HT turnover to a greater extent in neonates than in older animals (Tissari, 1975; Tissari & Tikkanen, 1978). Such an increase in 5HT synthesis and release induced by food deprivation may be of functional significance in inducing suckling behavior in the neonate since there are ample postsynaptic substrates for 5HT even in the neonate.

The first experiment investigated the influence of 24 hour food and/or maternal deprivation and ambient temperature on levels of plasma NEFA and 5HT and its metabolite 5-hydroxyindole acetic acid (5HIAA) in various brain regions of 3, 9, 15 and 21 day old rat pups. Food deprivation at nest temperature increased 5HT and 5HIAA levels in 3 and 9 day, but not 15 or 21 day old animals. These increases were not related to alterations in serum NEFA's; NEFA's were decreased by deprivation in neonates and conversely increased in weanlings. The second experiment investigated the effects of 2, 4, 16 and 24 hours of food deprivation on 5HT and 5HIAA in various brain regions of 3 and 9 day old pups. By 16 hours of deprivation, increased levels of 5HT and 5HIAA were observed, an effect that was most dramatic at 3 days of age. These results suggest that the NEFA-independent deprivation-sensitive nature of the serotonergic system in neonates is not characteristic of older animals, evidence consistent with our hypothesis that 5HT may be important for stimulating suckling in neonates while inhibiting it in older rat pups.

276.13 **NORADRENALINE-DEPLETION DURING EARLY DEVELOPMENT INHIBITS CORTICAL RESPONSE TO ENVIRONMENTAL EXPERIENCE.** M.Mirmiran*, E.Brenner* and H.M.M.Uylings*, (SPON: European Neuroscience Association), Netherlands Institute for Brain Research, Amsterdam, Netherlands.

Post-weaning differential environmental experiences have been found to be effective in influencing the growth of the cerebral cortex in rats. Pre-weaning suppression of REM-sleep by means of chronic clonidine (an α-noradrenaline agonist) treatment was found to counteract the post-weaning enrichment effects on cortical growth *(1)*. The present study was undertaken in order to determine whether REM-sleep or central noradrenaline neurotransmission, both of which are being affected by clonidine, is the crucial factor in inhibiting the environmental effect on cerebral cortex.

Male Wistar rat pups were injected subcutaneously with either 100 mg/kg 6-OH-DA or saline on days 1,3,5, and 7 after birth. The drug regimen did not significantly influence the sleep-wake pattern of the developing rats. After weaning both saline and 6-OH-DA injected animals were reared under either 'enriched' or 'standard' environmental conditions. At 75 days of age the brains were dissected into 8 parts and weighed immediately. A specific increase was found in the weight of the cerebral cortex in 'saline-enriched' *vs.* 'saline-standard' groups. This environmental difference was absent between the two neonatally 6-OH-DA injected groups. Furthermore, a significant reduction in cortical growth was found in both 'standard' and 'enriched' 6-OH-DA treated animals.

These results, together with clonidine results *(1)* suggest that noradrenaline neurotransmission during early development is an important factor in mediating cerebral cortical growth.

(1) M.Mirmiran, H.B.M.Uylings and M.A.Corner: Pharmacological suppression of REM sleep prior to weaning counteracts the effectiveness of subsequent environmental enrichment on cortical growth in rats. Dev. Brain Res. *7* (1983) 102-105.

276.14 MODIFICATION OF RECEPTOR BINDING IN CAT VISUAL CORTEX DURING DEVELOPMENT: CORRELATION WITH FUTURE PLASTICITY. C. Shaw[1], C. Aoki[3], M. Wilkinson[2] and M. Cynader[1,2]. [1]Dept. of Psychology and [2]Dept. of Physiology and Biophysics, Dalhousie University, [3]Laboratory of Philip Siekevitz, Rockefeller University.

Receptor binding sites play a crucial role in neural signal transmission and modulation. Yet despite the wealth of information on normal neural properties and their modification by restricted visual experience, very little is known about the types or characteristics of receptor binding sites in cat visual cortex. We examined various receptor binding sites in cat visual cortex in terms of ontogenesis of receptor characteristics and the effects of dark rearing. Previous studies showed that the β-adrenoceptor antagonist, ^{3}H-dihydroalprendol labelled a single binding site (K_D = 0.54 ± 0.04 nM) at all ages (Wilkinson et al., 1983). The developmental curve showed a peak in the total number of receptor binding sites (B_{max}) by 3 mo. of age. Dark rearing had no effect on either K_D or B_{max}.

To examine benzodiazepine receptors, male colony cats were sacrificed at different ages. Benzodiazepine (BZD) receptor sites were examined in washed homogenates of visual cortex. BZD binding was assayed using ^{3}H-flunitrazepam (87.3 Ci/mmole), a BZD agonist, with clonazepam (3 x 10^{-6}M) used to measure non-specific binding. Incubations were performed at 0°C for 75 min., followed by filtration through Whatman GF/B filters and two washings with cold Tris buffer. All assays were run in triplicate. ^{3}H-flunitrazepam labelled different affinity binding sites at different ages in normal cats (3 day, K_D = 1.49 ± 0.18 nM; 57 day, 4.2 ± 0.48 nM; adults, 2.51 ± 0.40 nM). The B_{max} rose rapidly from birth, peaked at 2 mo. postnatal, and declined slightly thereafter. The nature of the BZD-GABA complex is not fully understood, but it appears that GABA can alter the characteristics of BZD binding. Addition of GABA (10^{-4}M) to homogenates of visual cortex in normal cats induced large increases in BZD affinity, but had little effect on B_{max}. The magnitude of the GABA effect on K_D varied with age (3d, 2.3x; 57d, 1.4x, adult, 2.8x). Dark rearing had no effect on B_{max}, but affinities were higher except in adult animals. GABA mediation of BZD binding was much lower in the adult dark-reared cats (1.7x) than normal adults. The latter observation may reflect alternations in the binding characteristics of GABA receptors (see Needler et al., 1983, this volume).

BZD receptor affinities thus changed with age, with addition of GABA, and following dark rearing. These data suggest that dark-reared cats retain greater plasticity (Cynader & Mitchell, 1980) than their normal counterparts by an alteration in the development of certain receptor binding characteristics.

276.15 ONTOGENY AND LAMINAR DISTRIBUTION OF GABA RECEPTOR SITES IN THE STRIATE CORTEX OF NORMAL AND DARK REARED CATS. M. C. Needler*, C. Shaw and M. Cynader (SPON: V. LoLordo). Dept of Psychology, Dalhousie Univ., Halifax, N.S.

Gamma-aminobutyric acid (GABA) is thought to be a major inhibitory neurotransmitter in the cat visual cortex. Little is known, however, about the ontogeny, total number, affinity or distribution of GABA receptor binding sites. We have used [^{3}H]-muscimol (29.4 Ci/mmole), a potent GABA agonist, to autoradiographically localize GABA receptor sites in slide-mounted sections (Young and Kuhar, 1979; Penney et. al., 1981) of the striate cortices of normal and dark reared cats. Animals were perfused with PBS, their brains rapidly removed and frozen. After three rinses in cold Tris citrate to remove endogenous GABA, selected sections were incubated with [^{3}H]-muscimol for 30 minutes at 4°C. In adjacent sections, non-specific binding was determined in the presence of 10^{-4}M GABA. Sections were rinsed in three five second washes in cold Tris citrate to remove unbound ligand. Sections were apposed to either dry emulsion-coated (Kodak NTB2) coverslips or LKB Ultrofilm.

Dense [^{3}H]-muscimol binding was observed in the striate cortices of all cats. The low (15%) nonspecific binding observed in sections which had been coincubated with GABA indicated that most of the [^{3}H]-muscimol binding involved specific receptor sites in the cortex. The number of these receptor sites was relatively low (60% of peak density) in a 3-day old kitten, increased with age until 1-2 months postnatally, and then declined to about 70% of this peak value in adulthood. A clear laminar distribution of receptor sites was observeable at all ages. [^{3}H]-muscimol binding increased gradually from layer I to layer III, peaked dramatically in layer IV, fell of sharply in layer V and increased again in layer VI. These laminar differences were least striking in very young kittens.

The ontogeny and laminar distribution of GABA receptor sites in dark reared cats mirrored our findings for normal animals. Dark reared cats, however, demonstrated consistently higher levels of labelling than did normal animals of comparable ages. Experiments now in progress will determine whether this difference reflects changes in total number (B_{max}) or in binding affinities of the receptor sites. These findings parallel those for benzodiazepine receptor sites (Shaw et. al., 1983, this volume). The results suggest that GABA receptor sites can be modified by visual experience, and may thus be involved with some of the physiologic alterations observed in the visual cortex of dark reared animals.

276.16 DEVELOPMENTAL AND NEURONAL REGULATION OF β-ADRENERGICALLY-ACTIVATED ADENYLATE CYCLASE ACTIVITY IN THE RAT PAROTID GLAND. Barbara R. Talamo , Neurosciences Labs, Dept. of Neurology, Tufts Medical School, Boston, MA 02111.

β-adrenergic activation of adenylate cyclase activity in the rat parotid gland increases sharply between 14 and 18 d postnatal. This increase does not correlate with changes in β-adrenergic receptor density [Ludford and Talamo, J. Biol. Chem. 255:4619 (1980)], but parallels an increase in nucleotide binding protein (G/F) detected in membranes by covalent labeling with ^{32}P-NAD in the presence of cholera toxin [Ludford and Talamo, J. Biol. Chem. 258:4831 (1983)]. The two proteins labeled by cholera toxin, of M_r 43,000 and 48,000, increase two-fold during this critical 4 day period while [^{125}I]-HYP binding and affinity remain unchanged. During this same period, large increases in fluoride activation also occur, supporting the hypothesis that the increased response is due, at least in part, to increases in G/F. In rats denervated neonatally with 6-hydroxydopamine, β-adrenergic receptor sites develop at a normal rate and increase only after maturation of the cells is normally completed. The 1.5 fold increase in receptor density is accompanied by a 1.7 fold decrease in K_{act} of isoproterenol for adenylate cyclase, but maximal stimulation of adenylate cyclase activity does not increase. There is no change in the amount of G/F. Another explanation for the developmental increase in hormone-activation of adenylate cyclase could be that the catalytic unit (C) of the cyclase itself is increasing. C was assayed by activation with forskolin, reported to activate C in S-49 lymphoma cells, which have no detectable G/F by cholera toxin labelling. Forskolin activation is not enhanced by addition of guanine nucleotides. Stimulation is 21-fold at maximal levels of 700 M forskolin and is greater in Mn^{++} than in Mg^{++}. Low concentrations of forskolin potentiate -adrenergic activation of cyclase in parotid membranes in the presence of Mn^{++}, which by itself gives only a 3-fold stimulation. This indicates some enhancement of the receptor-G/F unit interaction with C in the presence of forskolin. However, maximal NaF activation, which proceeds through G/F, is additive with maximal forskolin stimulation. This suggests that G/F-sensitive cyclase and forskolin-sensitive cyclase may comprise different populations. Preliminary results show that maximal levels of isoproterenol stimulation in mature animals increase 2.4-fold compared to membranes prepared from 10d animals , while NaF stimulation increases 2-fold and isoproterenol + limiting forskolin increases by 2.3-fold. Maximal forskolin stimulation increases only 1.5-fold, suggesting that increases in C are less than increases in G/F.

276.17 ONTOGENY OF α_1-RECEPTORS AND THE EFFECT OF CASTRATION ON α_1-RECEPTOR LEVELS IN MALE GUINEA PIGS. A.E. Johnson*, B. Nock*, H.I. Ryer*, and H.H. Feder* (SPON: E. Satinoff) Institute of Animal Behavior, Rutgers Univ., Newark, NJ 07102.

In order to describe the development of α_1-receptors in male guinea pig brain, four different brain regions known to contain a high concentration of α_1-receptors (cortex) or steroid receptors (preoptic area, hypothalamus, and amygdala) were assayed at various stages of prepubertal development (1, 7, 14, 21 days) and in adulthood (60 days) using 1.25nM (^{3}H)prazosin. We found that (^{3}H)prazosin binding in cortex remained low (66% adult levels) through postnatal day 14 then gradually increased to adult levels. Binding in POA and HYP decreased from day 1 (132% and 116% of adult) to adult levels by day 7 in HYP and day 21 in POA. No developmental changes were found in AMG. Because differences in (^{3}H)prazosin binding demonstrated above could be due to changes in apparent affinity (Kd) or receptor number (Bmax), α_1-receptors were assayed using a range of concentrations of (^{3}H)prazosin (.1 to 1.0nM). Analysis of Rosenthal plots showed that differences in binding between infants (1 to 3 days old) and adults were due to changes in receptor number rather than affinity.

Since gonadal activity appears to increase during development and steroids have been shown to influence receptor levels in other neurotransmitter systems we examined the effects of endogenous steroids on α_1-receptors. We tested the effects of neonatal or adult castration on α_1-receptor content in CTX, POA, HYP, and AMG of male guinea pigs. No differences were found between castrated (either neonatal or adult) and intact animals. These results agree with the failure to demonstrate an effect of estrogen and progesterone on α_1-receptor levels in the female guinea pig (Nock and Feder, 1983). However, since receptors were measured in relatively large brain areas, possible steroid effects on discrete nuclei might be obscured. Therefore, steroid regulation of α_1-receptors cannot be ruled out at this time.

DEVELOPMENT AND PLASTICITY: LIMBIC SYSTEM

277.1 BOTH TRANSITORY AND IRREVERSIBLE FIMBRIA/FORNIX "CONDITIONING" LESIONS FACILITATE RECOVERY FROM BILATERAL HIPPOCAMPAL LESIONS IN RATS. B.E. WILL and G. TONIOLO*. Lab. de Neurobiologie des comportements, Université Louis Pasteur, 67000 Strasbourg, France.

The aim of the present series of experiments was to evaluate whether a cerebral "conditioning" lesion could facilitate recovery of behavioral function following a second or "testing" lesion in rats. The "conditioning" lesions used were unilateral fimbria/fornix lesions, produced either by electrolytic or transitory chemical (colchicine 5µg, 10µg, 15µg) treatment . The "testing" lesions were one-stage electrolytic bilateral dorsal hippocampal lesions. One group of rats (H) sustained only hippocampal lesions without any "preconditioning" ; two groups of unlesioned rats served as controls (one injected with buffer and one with electrode intrusion). Hebb-Williams maze learning was conducted one month after hippocampal lesions and two months after "preconditioning". In comparison with controls, a significant increase in errors was observed with both the H and the "preconditioned" groups. However, in comparison to the H rats, those "preconditioned" by electrolytic as well as 5µg colchicine lesions performed at a higher level. The colchicine effects were dose dependent. The AChE and CAT activities were determined in the hippocampus. The biochemical results are discussed in relationship to the behavioral results emphasizing their importance for the understanding of the serial lesion effect.

277.2 HIPPOCAMPAL-CHOLINERGIC SYSTEM DEVELOPMENT AND PLASTICITY IN NORMAL AND EARLY LEAD EXPOSED RATS. D. P. Alfano, T. L. Petit and J. C. LeBoutillier*. Div. Life Sciences and Dept. Psychology, Univ. of Toronto, West Hill, Ont. M1C 1A4.

A review of previous evidence suggested the possibility of a functional association between the effects of early lead (Pb) exposure, hippocampal damage and cholinergic deficiency. To further assess this possibility, Long-Evans hooded rat pups were exposed to Pb for the first 25 postnatal days via the maternal milk. Dams were fed either 4.0% $PbCO_3$ or a Na_2CO_3 control diet throughout this period. At 30 and 115 days of age, the brains of Pb and control animals were processed for acetylcholinesterase histochemistry. Morphometric evaluation of the molecular layer of the hippocampal dentate gyrus indicated that while absolute increases in the dimensions of the afferent systems to the hippocampal dentate gyrus are observed between 30 and 115 days of age, no significant rearrangement in the pattern of lamination occurs during this time. No effects of Pb were seen on the development of the cholinergic innervation of this brain region at either of these ages. Unilateral perforant path transections performed on Pb and control animals at 100 days of age indicated reduced cholinergic plasticity in the molecular layer of the hippocampal dentate gyrus of Pb exposed animals, as indicated by AChE histochemistry. These findings indicate that a decrease in neuroanatomical plasticity may be a critical brain mechanism underlying the learning deficits observed following exposure to Pb.

277.3 MESOLIMBIC DOPAMINERGIC MODULATION OF THE EXCITATORY RESPONSES OF THE NUCLEUS ACCUMBENS NEURONS TO HIPPOCAMPAL STIMULATION. C. R. Yang* and G. J. Mogenson. Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

The nucleus accumbens (NAc) of the ventral striatum receives converging inputs from many limbic regions, including afferents from the subiculum of the hippocampus (Hipp). This nucleus also receives a rich dopaminergic (DA) projection from the ventral tegmental area (VTA). In earlier experiments using extracellular single unit recording techniques, we observed that the excitatory responses of the NAc neurons to single pulse stimulation of the Hipp (subiculum) were attenuated by a conditioning train of stimulus pulses (10 Hz) delivered to the VTA (Yang and Mogenson, Can.Fed.Biol.Soc.abs.,1983). This attenuation did not occur in animals in which the VTA was treated two days earlier with 6-hydroxydopamine (with desipramine pretreatment). These observations suggest that the attenuation reaction might be mediated by the mesolimbic DA neurons. Thus, the present study was undertaken to obtain further evidence to show whether the attenuation, resulting from VTA conditioning stimulation, of the activation of NAc neurons to Hipp stimulation is dopamine-mediated.

Extracellular single unit recordings were obtained from the medial NAc of urethane-anaesthetized rats using glass micropipettes filled with 0.2% pontamine sky-blue in 0.5 M sodium acetate. In some experiments, a recording electrode filled with 3M NaCl and assembled in parallel with a five-barrelled micropipette was used for simultaneous recording and iontophoretic application of DA.

In the first series of experiments in 8 rats haloperidol, a DA antagonist, was injected intraperitoneally (0.3-0.5 mg/kg) to investigate the attenuation effect on the activation of the NAc neurons to Hipp stimulation resulting from VTA stimulation. Following haloperidol administration, the attenuation previously observed with VTA stimulation occurred in only 39% (23/59) of the NAc neurons tested ($\chi^2 = 35.9$, $p < 0.001$).

In a second series of experiments in 8 rats DA was administered by iontophoresis to NAc neurons activated by Hipp stimulation. The iontophoresis of DA(5-20 nA) significantly reduced the Hipp activation of 89% (25/28) of the NAc neurons tested. This effect is similar to the attenuation effect which resulted from VTA stimulation.

The observations suggest that the excitatory inputs of Hipp to NAc neurons are modulated by the mesolimbic dopaminergic system.

(Supported by the Medical Research Council of Canada)

277.4 SEX DIFFERENCES IN THE DENDRITIC RESPONSE TO DIFFERENTIAL ENVIRONMENTS IN THE GRANULE CELLS OF THE DENTATE GYRUS. Janice M. Juraska, Jonathan Fitch*, Constance Henderson*, and Natalie Rivers*. Dept. of Psychology, Indiana Univ., Bloomington, IN 47405.

Males have often been described as the more vulnerable sex and, therefore, may be more susceptible to the effects of their rearing environment. We have previously found that male rats show greater dendritic differences than female rats in some cortical neuronal populations following rearing in complex (EC) and isolated (IC) environments.

The dentate gyrus of the hippocampus is a late developing structure that is likely to be susceptible to environmental effects. We reared male and female hooded rats in either EC or IC for a month after weaning in two separate replications. Granule neurons were sampled from the upper one-third and the lower two-thirds of the granule cell layer in Golgi-Cox stained coronal sections. A concentric ring analysis revealed that in both replications in both upper and lower layers neurons from females showed large differences due to the environment with EC females having more dendritic material in the middle to upper dendritic tree than IC females. Male differences in response to the environment were very small to nonexistent. An analysis of branch number and length in the first replication revealed that this female plasticity occurred in terminal branch length. Evidently the type of sex differences seen in dendritic response to environmental conditions varies with the area of the brain examined. In the dentate, females show much larger dendritic *differences than males.*

Supported by NIH HD14949 and the MacArthur Foundation.

277.5 DEVELOPMENT OF THE GABA SYSTEM IN THE RAT HIPPOCAMPUS. D. L. Martin, and J. W. Swann. (SPON: A.T. Campagnoni). Center for Labs and Research, NYS Dept. of Health, Albany, NY 12201.

Previous studies in our laboratory have shown that the CA3 region of hippocampal slices from immature rats (9-19 days old) has a pronounced capacity to generate prolonged, self-sustained afterdischarges in response to brief repetitive orthodromic stimulation (Swann and Brady, Neurosci. Abst. (1982) 8, 1016). Since GABA-mediated inhibitory synaptic transmission is thought to play a central role in the prevention of epileptogenesis in mature hippocampus, we have examined the development of the hippocampal GABA system. Glutamate decarboxylase (GAD) activity in whole hippocampus and in the CA1, CA3, and area dentata (AD) subfields was measured as a function of age. Intracellular recording was also used to determine the presence of GABA-mediated ipsps in immature hippocampus.

Whole hippocampi were dissected, blotted, weighed and frozen at 3, 10, 13, 16, 20, 23, 30 and 112 days postnatal. The CA1, CA3 and AD regions were dissected, weighed and frozen from animals killed at 10, 16 and 30 days. GAD activity was determined in homogenates of the tissue samples by measuring the production of [^{14}C]GABA from uniformly labeled [^{14}C]glutamate. Total hippocampal GAD activities at 3 and 10 days were only about 2 and 14 percent of the activity in mature hippocampus (2.1 µmol GABA/h/hippocampus at 30 days). Thus about 86% of the GABA system developed after 10 days of age. The specific activity of GAD increased about 3 fold (from 1.6 to 4.7 nmol/h/mg tissue) from 3 to 10 days and approximately tripled again by 20 days (15.1) after which there was little further increase. This pattern is similar to that observed in other brain regions. The developmental patterns of GAD in CA1, CA3 and AD were parallel with small but significant differences in the specific activity of GAD among the subfields. The specific activity of GAD approximately tripled between 10 and 30 days in all three subfields. Intracellular recordings revealed that orthodromic stimulation of the CA3 region of day 10 hippocampal slices resulted in brief monosynaptic epsps followed by prolonged ipsps which were similar in appearance to those in mature CA3 pyramidal cells. Moreover, bath application of the GABA antagonists bicuculline or penicillin produced ipsp blockade and depolarizing shift generation in the immature pyramidal cells. Thus, inhibitory Gabergic synaptic transmission appears to be functional at an age when GAD activity is only about 14% of that in the mature hippocampus. Pre- or postsynaptic alterations in the efficacy of GABA synapses during repetitive stimulation may be responsible for the seizure sensitivity of the immature hippocampus.

Supported by grants NS-18309 and MH-35664 from USPHS/DHHS.

277.6 VARIATIONS IN THE CYTOARCHITECTURE OF THE DENTATE GYRUS OF THE SPRAGUE-DAWLEY RAT. D.R. Pierce and J.R. West. Dept. of Anatomy, Univ. of Iowa, College of Medicine, Iowa City, IA 52242.

The relatively simple cytological organization of the hippocampal formation lends itself well for studying a variety of basic topics of neurobiology including development and neuronal plasticity. Although long thought to possess uniform organization throughout its length, it is now recognized that some variations exist along the septotemporal axis. There are cytological variations in the dentate gyrus of adult albino rats. Sprague-Dawley rats from two sources (King Labs, Oregon, WI and Harlan Sprague-Dawley, Indianapolis, IN) exhibit major distortions in the form of convoluted dentate gyri and a wider, flattened hilar region in the temporal one-third of the hippocampal formation. In horizontal sections the distortions appeared as an accessory dentate gyrus. In coronal sections the variations ranged from slight ripples in the molecular layer to convolutions (major distortions) of the medial (infrapyramidal) blade of the dentate gyrus. Major distortions occurred in approximately half of the animals that we examined. They occurred in both males and females, were found unilaterally on either side, and occasionally were bilateral. In spite of the sometimes rather dramatic alterations in the shape of the medial blade, the molecular layer retained its characteristic trilaminarity in Timm's-stained sections. The distortions were not artifacts due to perfusion or sectioning; they were found in brains that were not perfused and in those sectioned in a variety of planes. These variations in organization may prove to be useful in the future for investigating the normal development of convoluted cortical structures.

This study was funded in part by grant AA05523 to J.R.W.

277.7 PERINATAL GLUCOCORTICOIDS ALTER DENTATE GYRUS EVOKED POTENTIALS. J. Vicedomini*, A. Nonneman, S.W. Scheff, and S.T. DeKosky. Psychology, Anatomy, and Neurology Depts., Univ. Kentucky and Lexington V.A. Medical Center, Lexington, KY 40506.

Perinatal glucocorticoid administration in rats retards postnatal neurogenesis in populations of dentate gyrus granule cells. The current study examined extracellular evoked potential activity in the dentate gyrus of adult rats given a single injection of dexamethasone early in ontogeny.

Rats received either a high dose (100 mg/kg) or low dose (1 mg/kg) of dexamethasone hydrochloride on postnatal day 4. One group of control subjects received an injection of saline; another control group, a nutritional control group, received maternal deprivation (8 hrs. daily access to dam) intended to produce body and brain growth suppression comparable to that observed in drug treated subjects. In adulthood we assessed various electrophysiological measures: laminar depth profiles, input/output functions, and paired-pulse facilitation.

Control and nutritional control subjects did not differ significantly from each other on any measure. Laminar depth profile analyses revealed significant reductions (30%) in the spatial extent of the negative field potential evoked in high and low dose subjects. The location of the major synaptic sink, produced by monosynaptic perforant path activation in stratum moleculare, was also significantly changed in high and low dose subjects. Analyses of input/output functions revealed that high dose subjects required significantly higher inputs to generate population synaptic potentials (PSPs, 26%) and population spikes (PSs, 28%) than those found in low dose and control subjects. These group differences emerged in tests using high intensity stimuli, as no significant differences in threshold (stimulus required to first generate PSP or PS activity) were found. Paired-pulse stimulation tests also revealed significant effects of the perinatal dexamethasone treatment. Maximal PSP facilitation was observed in control subjects using a 35 ms interstimulus interval (ISI). High dose and low dose subjects exhibited significantly less PSP facilitation at 35 ms. Low dose subjects failed to show significant PSP facilitation at any ISIs tested, while high dose subjects exhibited maximal PSP facilitation at ISIs longer (60 ms) than those found to be most effective in controls. These electrophysiological findings may underly the behavioral impairments observed in tests of hippocampal function reported for rats treated with glucocorticoids. (Supported by NIH grants NS16981, NS00444 and the V.A. Medical Research Service.)

277.8 BIRTHDATES OF NEURONS IN THE ENTORHINAL AND SUBICULAR CORTICES OF THE CAT. J. M. Wyss, B. Sripanidkulchai* and T. L. Hickey. Departments of Anatomy and Physiological Optics. Univ. of Alabama in Birmingham, Birmingham, AL 35294.

The [^{3}H] thymidine autoradiographic method was used to determine the birth dates of neurons in the cat parahippocampal gyrus. Cat fetuses were exposed to a single pulse of the radioactive marker between the 20th and 55th embryonic day. All animals were delivered normally and allowed to survive for 2-6 months postnatal. The resulting autoradiographs demonstrate three spatiotemporal gradients of cell birth in the entorhinal and subicular cortices. First, an inside-out gradient is apparent; i.e., neurons in the deeper layers are born earlier than those in the more superficial layers. Second, a rhino-to-dentate gradient exists. Accordingly, cells closer to the lateral entorhinal region tend to be generated earlier than those further away. Third, a temporal-to-septal gardient is present. Neurons close to the anterior pole of the temporal lobe are born earlier than those more caudally located. Whereas the first two gradients have been observed in other species, the latter gradient has not been reported consistently. Three exceptions to these overall gradients exist. First, neurons near the layer I/II border are born earlier than the majority of the layer II neurons. Second, neurons near the transition zone between two adjacent regions are born earlier than neurons located in the middle of each region. Third, the prosubiculum and subiculum do not exhibit a clear inside-out or temporal-to-septal gradient. These exceptions to the normal gradients may shed light on the processes underlying the normal alignment and migration of neurons.

Supported by N.I.H. Grants NS 16592, EY 01338 and EY 03039.

277.9 STRAIN DIFFERENCES IN CELL NUMBERS IN THE RAT DENTATE GYRUS. Gary M. Peterson and Barbara D. Boss. The Salk Institute, and the Clayton Foundation for Research/California Division, P.O. Box 85800, San Diego, CA 92138.

In the past decade there have been several papers that have included estimates of the numbers of granule cells in the rat dentate gyrus. The published figures for the total number of granule neurons on each side varies from just over 600,000 to over 2 million. Although the highest estimate is clearly erroneous (being based on inappropriate methods for estimating cell density), it is not clear why there should be so much variability in the reports from different laboratories. Bayer (1982) has recently suggested that much of the variability is attributable to the study of animals of different ages and her own counts of the numbers of dentate granule cells in Wistar rats indicate a progressive increase in the number of granule cells during the first year from just under 900,000 to 1.28 million (an increase of 40%). Since postnatal neurogenesis is a striking feature in the rodent dentate gyrus, this hypothesis is both plausible and attractive, but it is only partially supported by our own data. We report here a strain difference between Sprague-Dawley and Wistar rats in: (1) the volume of the granule cell layer and in the total number of granule cells; and (2) the changes in these two measures with age.

Volume and cell number was estimated from a series of Sprague-Dawley rats 1, 4, and 14 months of age and Wistar rats 1, 4, and 12 months of age, using the method described by Schlessinger et al. (1975). In Sprague-Dawley rats we have found no evidence for an increase in granule cell number between 1 and 14 months of age; the total number of cells ranged from 1.15 to 1.24 million. The volume of the granule cell layer also remained relatively constant at 1.14 to 1.20 mm^3. Cell density ranged from 9.6 to 10.9 x 10^5 granule cells/mm^3. In our 1 month old Wistar brains the volume and total cell number were substantially less than in the Sprague-Dawley brains of the same age. During the first year the volume of the granule cell layer in the Wistar animals increased from .76 mm^3 to 1.13 mm^3 (49%) and the number of granule cells increased from 640,000 to 810,000 (26%). The cell density was less than in age-matched Sprague-Dawleys and decreased slightly over one year from 8.4 to 7.1x10^5 cells/mm^3 (15%).

Comparing our data with that from other laboratories it would appear that there are significant strain differences not only in the total number of granule cells and in the volume of the cell layer, but also in the pattern of postnatal development. Using the same methods for cell counting and for estimating the volume of the dentate gyrus our data suggest that there is an increase of about 25% in the number of granule cells during the first year in Wistar rats but not in Sprague Dawley rats and that, on average, the number of granule cells in the Sprague Dawley dentate gyrus is somewhat higher than in Wistar rats.

Supported by NIH Grants NS-06867 and NS-16980.

278.1 EFFECTS OF CHOLECYSTOKININ ON INTRACELLULAR RECORDING IN CAUDATE NEURONS. E. Andersen, C. Hull and N. Buchwald, Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

Heavy concentrations of cholecystokinin (CCK) cell bodies are found in the rat cerebral cortex. The caudate nucleus (Cd) has been shown to contain high levels of CCK receptors. The prepiriform cortex (Cx) in the cat has been demonstrated with both anatomical and electrophysiological techniques to have a direct projection to the Cd. This presents the questions of whether there is a CCK pathway from Cx to Cd, what effect CCK has in the cat, and how CCK can be tested and manipulated. Intracellular recordings in the Cd were made in response to electrical stimulation of Cx and to direct application of CCK. CCK effects were compared to those of GABA, glutamate, and the CCK vehicle. Finally, CCK antibody was used to manipulate the effects of CCK.

The micropressure technique was utilized to directly apply the test substances to the Cd in cats. This method eliminates possible alterations of the peptide and antibody caused by the electrical currents used in traditional microiontophoretic methods. The ejection pipettes were constructed with 2-5 barrels of capillary tubing. The recording electrode was attached so that it projected 80-120μ from the ejection barrels.

Intracellular recordings showed that micropressure application of CCK octapeptide (0.1mM) onto single Cd neurons caused a large membrane depolarization of 30-50mV in 38/45 neurons, a dramatic increase in the firing rate in 29/45 neurons, and a decrease in the amplitudes of the postsynaptic potentials (PSPs) evoked by Cx stimulation in 9/11 neurons. In comparison, pressure application of glutamate caused membrane depolarizations of 10-30mV and an increased firing rate without affecting the PSPs, whereas GABA hyperpolarized the membrane, decreased the firing rate and also decreased the amplitudes of the PSPs. The onset of the effects occurred 2-15 sec after 1 kg/cm^2 pressure was applied and the effects outlasted the application period by 15-120 sec. The CCK vehicle, Bovine Serum Albumin, had no effect on 9/12 Cd neurons. Direct application of the CCK antibody blocked the effects of subsequent CCK applications in 6/8 Cd neurons.

These results are in accord with other recent studies which indicate that CCK may be used as a neurotransmitter in the CNS. Components of the excitatory effects of CCK in the Cd seem to be comparable to response components of both glutamate and GABA. There may be CCK projections from the Cx to the Cd in addition to CCK inputs from local Cd neurons. Finally, this study shows that peptide antibodies may be used to manipulate the electrophysiological effects of this class of neurotransmitters.

Supported by USPHS grants HD 05958 and HD 07032.

278.2 RAT NEOSTRIATAL NEURONS IN IN VITRO SLICE PREPARATIONS: (II) THE IONIC NATURE OF REGENERATIVE POTENTIALS. H. Kita, T. Kita* and S. T. Kitai. Dept. of Anatomy, Michigan State University, East Lansing, MI 48824.

The ionic nature of regenerative potentials in rat neostriatal neurons was studied using in vitro slice preparations. The procedures used to prepare neostriatal slices and the methods used for intracellular recordings and intracellular labeling were the same as in the accompanying abstract. Direct stimulation of the neuron with outward current injections through the recording electrode generated fast spike potentials (FSPs). FSPs were completely blocked by the application of TTX (10^{-5} g/ml) in Ringer solution. After application of TEA (2-5 mM), outward current injections could generate FSPs and slow spike potentials (SSPs). The SSPs were characterized by slow depolarizations, long durations and slow repolarizations. Application of TTX in TEA-containing Ringer solution blocked generation of FSPs but not SSPs. These SSPs were not triggered after the substitution of Ca^{++} by Mg^{++} in the medium. The above data suggested both Na^- and Ca^{++} spikes could be generated in neostriatal neurons. Depolarizing postsynaptic potentials (DPSP) evoked by relatively strong local stimulation triggered both FSP and SSP in some neurons. SSPs were more easily triggered by short interval double stimulations or a local stimulation during artificial depolarization of the neuron by outward current injections. After application of TEA, all the neurons triggered SSPs on DPSPs. The SSP on DPSPs were not seen after reduction of Ca^{++} concentration. The results that outward current injection into neurons in normal Ringer solution never generated SSPs, but DPSPs evoked by local stimulation could trigger SSPs, suggested tha the threshold for Ca^{++} spikes was lower in dendrites (where most excitatory synapses are found) than somata. The SSPs may serve an important function in the integration of synaptic inputs onto neostriatal neurons and may trigger Ca^{++} dependent phenomena such as a Ca^{++} induced K^+ permeability increase.

(Supported by NIH Grant NS 14866 to S.T.K.).

278.3 RAT NEOSTRIATAL NEURONS IN IN VITRO SLICE PREPARATIONS: (I) ELECTRICAL MEMBRANE PROPERTIES AND LOCAL STIMULATION EVOKED RESPONSES. S. T. Kitai, H. Kita and T. Kita*, Dept. of Anatomy, Michigan State University, East Lansing, MI 48824.

Electrical membrane properties and responses to local stimulation were studied in in vitro slice preparations. Rats were decapitated, the brains were removed and blocks containing the neostriatum were sectioned on a Vibratome (250-400 μm) in the parasagittal plane. A slice was placed in a recording chamber and continuously superfused by oxygenated Ringer solution (concentration in mM: NaCl 124, KCl 5.0, $MgSO_4$ 2.0, KH_2PO_4 1.25, $CaCl_2$ 2.0, $NaHCO_3$ 26 and glucose 10, pH 7.2-7.4). Local stimulation was applied through a bipolar electrode placed on the surface of the slice. Glass pipettes filled with 2M K-methylsulfate or 3-4% HRP in 0.05 M tris buffer (pH 7.6) and 0.5M K-methylsulfate with the DC resistance between 30-70 MΩ were used for recordings.

The data reported here are only from neurons having stable resting membrane potentials of more than 50 mV which were to generate spike potentials of amplitude greater than 70 mV. All neurons (n = 52) showed nonlinearity of input resistance in the hyperpolarizing direction (i.e. anomalous rectification). The mean input resistance at the resting membrane potential was 16.6 MΩ. The mean membrane time constant (τ_o) was 5.3 ms and derived from the semilogarithmic plots of transmembrane potential shift produced by small hyperpolarizing current pulses. In some neurons, the equalizing term (τ_1) was also determined and it has a mean value of 1.0 ms. Measurement of τ_o using the strength-latency relation showed a similar value (5.0 ms) to that measured from voltage transients. Direct stimulation of the neuron by outward current injections triggered a single fast spike potential (FSP) or repetitive FSPs. Strong outward currents decreased the amplitude and increased the duration of repetitive FSPs. For their increases in current strength resulted in inactivation of FSPs. Local stimulation evoked only depolarizing postsynaptic responses (DPSPs). Potentiation of DPSPs for longer trains of repetitive stimulation caused post-tetanic potentiations lasting for a few min. DPSPs may be compounded EPSPs and IPSPs, since both Cl^- injection into the neurons and bicuculline or picrotoxine application into the medium resulted in an increase in amplitude in DPSPs. Antidromic spikes preceding DPSPs were observed in some neurons after local stimulation. Local stimulation failed to trigger initial segment and somatic spikes when it was applied during DPSPs which were evoked by a preceding stimulation or during artificial membrane hyperpolarization. Intracellular labeling of neurons indicate that recordings were made from medium spiny neurons.

(Supported by NIH Grant NS 14866 to S.T.K.).

278.4 RAT NEOSTRIATAL NEURONS IN IN VITRO SLICE PREPARATION: (III) THE EFFECTS OF 4-AMINOPYRIDINE ON THE NEOSTRIATAL NEURONS. T. Kita*, H. Kita and S. T. Kitai (SPON: A. Koestner), Dept. of Anatomy, Michigan State University, East Lansing, MI 48824.

4-Aminopyridine (4-AP) which is known to broaden presynaptic spikes by blocking the repolarizing potassium flux and potentiate transmitter release has been used to enhance IPSPs in some in vitro studies of the CNS.

In this study, we observed the effects of 4-AP (0.1 M-1 M) on either synaptically or directly activated responses of rat neostriatal neurones in slice preparations.

1) Effects of 4-AP on synaptic responses evoked by local stimulation. Local stimulation evoked depolarizing postsynaptic potentials (DPSPs) with spikes. 4-AP prolonged the decay phase of DPSPs resulting in a long plateau which accompanied a conductance increase. The amplitude and polarity of the plateau were dependent on the membrane potential. The potentiation of DPSPs, observed with a paired stimulation in control medium was not seen after application of 4-AP. The amplitude of the plateau depolarization was potentiated by an intracellular Cl^- injection or in the presence of the low Cl^- medium. This potentiated plateau depolarization was decreased or blocked by administration of bicuculline or picrotoxin. Repetitive stimulation decreased the amplitude and half-decay time of the plateau depolarization. 4-AP application at times induced spontaneous activities which were never observed in normal slice preparation. Spontaneous activities were more clearly visible when the neuron was either injected Cl^- or when Cl^- was added to the medium.

2) Effects of 4-AP on the direct activation. After application of 4-AP, intracellular stimulation of the neuron with outward current injections generated fast Na spikes and slow spike potentials (SSPs). SSPs were characterized by a slow depolarization, long duration and slow repolarization. Addition of TTX (10^{-5} g/ml) in 4-AP containing medium blocked generation of fast Na spikes, but not SSPs. These SSPs were abolished after the substitution of Ca^{++} by Mg^{++} in the medium and with 1 mM EGTA.

These data suggested that 4-AP enhances synaptic transmission by an increase of Cl^- permeability. The effects of bicuculline or picrotoxin on the potentiation phenomena indicate that GABAergic inputs may influence Cl^- permeability. SSP generation by outward current injections in the presence of 4-AP would suggest that 4-AP may affect not only the presynaptic, but also the postsynaptic sites.

(Supported by NIH Grant NS 14866 to S.T.K.).

278.5 DEVELOPMENT OF SOMATOSENSORY RESPONSIVENESS IN THE BASAL GANGLIA IN THE CAT. M.S. Levine, J.S. Schneider, C.D. Hull, and N.A. Buchwald. Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

Our laboratories have shown that many of the connections of the basal ganglia (BG) are in place and functional by the first few postnatal weeks in the cat. There are, however, a number of indices that remain immature during early postnatal periods. The present experiments sought to ascertain further functional development of BG neurons in awake kittens prepared for chronic recording by determining responses of these neurons to tactile stimulation of the face (punctate or brushing stimuli). Neuronal responses in the caudate nucleus (Cd), the globus pallidus (GP), or entopeduncular nucleus (Ento) were assessed in developing kittens and compared with similar data obtained from adult cats. 522 Cd units were recorded in 7 kittens and adult cats. In the youngest (13-20 days) no cells (0/110) responded to facial tactile stimulation. In kittens 30-35 days old, 1% (1/101) of Cd cells tested responded to facial stimulation. This cell had a large receptive field (i.e., entire face), responded to both touch and brushing stimuli, but was not directionally sensitive. 6% (5/79) of Cd cells in 60-64 day kittens responded to facial stimulation. Responses occurred to brushing and were mostly nondirectional. 9% (6/67) of Cd cells in 90-100 day old kittens responded. Units directionally sensitive to brushing stimuli began to appear at this age. In contrast, in adult cats there were more responsive cells (22%, 36/162), more punctate responses with small receptive fields, and more directionally sensitive cells. To date, 124 GP cells and 89 Ento cells have been recorded from 6 kittens. In 30-40 day kittens 2% (1/40) of GP cells responded to facial stimulation, while 6% (1/17) of Ento cells responded. Receptive fields were the entire face, and responses were only to brushing (no directional sensitivity). In 60-70 day kittens, 12% (7/60) of GP cells and 7% (2/30) of Ento cells responded. Receptive fields were mostly bilateral and moderate to large in size. These cells responded almost exclusively to brushing stimuli and showed virtually no directional sensitivity. 33% (8/24) of GP cells and 19% (8/42) of Ento cells in 90-100 day kittens responded. Units responsive to punctate stimuli and those directionally sensitive to brushing stimuli appear at this age. The results contrast with those found in adult cats, where there are more responsive cells, more discrete receptive fields, and more punctate responses. These findings demonstrate both quantitative and qualitative maturational changes in the ability of BG neurons to encode sensory information during postnatal development.

Supported by USPHS grants HD 05958 and HD 07032.

278.6 BASAL GANGLIA INFLUENCES ON BRAINSTEM NEURONS. N.A. Buchwald, J.S. Schneider, M.S. Levine, and C.D. Hull. Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

There is increasing evidence that basal ganglia (BG) neurons can interact with both sensory and motor neurons in the brainstem. The present experiment was designed to characterize the electrophysiological interactions of BG output neurons and neurons in the trigeminal nuclei and adjacent brainstem areas.

Extracellular unit recordings were obtained from 8 adult cats. Twenty-three cells were sampled from the trigeminal main sensory nucleus (Sens. V), 10 cells from the trigeminal motor nucleus (Mot. V), 25 cells from the pedunculopontine nucleus (PPN), and 10 cells from the reticular formation (RF). 55% of Sens. V cells responded to stimulation of the trigeminal sensory ganglion (V gang.) only (mean latency=3 msec, s.d.=.08). 23% of Sens. V cells responded to both ganglion and entopeduncular nucleus (Ento) stimulation (mean latency=20 msec, s.d.=5.2). 40% of these cells, when tested in a conditioning-test pulse (C-T) paradigm, showed Ento inhibition of trigeminal sensory responses (effective C-T intervals ranging between 20-100 msec). Similar findings were obtained for RF neurons.

Twenty-two percent of PPN cells tested responded to V ganglion stimulation only (mean latency=12 msec, s.d.=2.0) while 25% responded to Ento stimulation only (mean latency=8 msec, s.d.=3.0) and 16% responded to both V ganglion and Ento stimulation. 5 PPN cells tested in a C-T paradigm showed Ento inhibition of evoked trigeminal sensory responses, with effective C-T intervals ranging between 50-125 msec.

In contrast to the effects upon Sens. V, RF, and PPN cells, no BG influences have been observed to date on neurons in the trigeminal motor nucleus. Many of the motor neurons tested responded to stimulation of the V ganglion and/or stimulation of the trigeminal mesencephalic nucleus. None of these cells responded to BG stimulation nor could trigeminal sensory responses be modulated by BG stimulation. At present, only masseter motor neurons have been identified (by antidromic response to masseter nerve stimulation). BG effects on digastric motoneurons are presently under investigation.

The data indicate that interactions exist between the BG, trigeminal system, and other brainstem areas. Of particular interest is the finding that trigeminal sensory and PPN neurons can be affected by BG stimulation while trigeminal motoneurons are apparently unaffected. These results suggest that previously observed BG effects on the trigeminal motor system may be mediated indirectly via influences on trigeminal sensory and RF neurons.

Supported by USPHS grants HD 05958 and HD 07032.

278.7 THE EFFECTS OF CONTEXT AND STIMULUS SPECIFICITY ON CAUDATE UNIT ACTIVITY DURING HEAD MOVEMENT. C. Manetto* and T.I. Lidsky, SUNY, Stony Brook, NY 11794. Many of the motor problems experienced by humans and experimental animals with basal ganglia (BG) damage are "context dependent" (i.e., these symptoms are present only during certain circumstances). For example, rats with lesions in the globus pallidus have difficulty positioning their heads during ingestion but show normal head positioning during grooming, walking, and rearing. Context dependent problems such as these seem to be characteristic of BG damage in general as limb and trunk movements are similarly affected.

Previous electrophysiological studies have implicated the caudate in the control of head movements (1). To assess the context dependent role of the BG in head movement, units in the caudate were recorded extracellularly in awake cats restrained in a device designed to allow horizontal rotational head movements. Simultaneously, EMG was recorded from the several neck and trunk muscles.

It was possible to elicit specific types of head movements by presenting various stimuli to the animals. For example, cats would track stimuli they found pleasurable (e.g, cat food) and avoid or move away from stimuli they found aversive (e.g., cod liver oil). Movements made in the absence of a distinct stimulus were categorized as spontaneous movements.

Forty cells, established as head movement related, showed clear changes in firing rate with sensory triggered movements. However, units showing activity with movements elicited by one stimulus showed either no response or a different response with movements of similar topography evoked by another stimulus. It should be noted that these unit responses were not sensory responses: on trials where stimulus presentation resulted in no head movement, there was no change in unit firing rate. None of the units showing head movement related activity responded during spontaneous movements.

These data suggest that the BG is involved in sensory-controlled movements rather than movement in general. This involvement is analogous to the supplementary motor area's (SMA) involvement in movement. Tanji & Kurata (2) demonstrated that units in SMA, responsive to sensory triggered movement, show stimulus specificity as well. It is noteworthy that this area may influence movement by gating sensory input into motor areas (3). Similar suggestions have been made about the mechanism by which the BG influence motor functioning (4). The anatomical connections from the BG to the SMA via VA/VL lend further support to the notion of BG gating of sensory information into motor systems (supported by NS 16054).

1. C. Manetto and T.I. Lidsky, in press.
2. J. Tanji and K. Kurata, J. Neurophysiol 1982, 48: 633-653.
3. T. Tanji and K. Taniguchi, J. Physiol, Paris 1978 74: 317-318.
4. J. S. Schneider et al., Exp.Neurol. 1982, 77: 534-543.

278.8 REDUCTION OF CORTICALLY EVOKED UNIT ACTIVITY IN RAT STRIATUM DURING TREADMILL LOCOMOTION. D.J. Woodward, M.O. West and J.K. Chapin. Dept. Cell Biology, University of Texas Health Science Center, Dallas, Texas 75235.

One of the objectives in studying the structure and function of the striatum is to clarify how convergent sensory and motor information is processed. In order to consider this issue, we have examined the responses of striatal neurons to electrical stimulation of neocortical areas during different motor behaviors. Adult, female Long-Evans rats were prepared for chronic recording with a detachable microdrive that was located over the striatum, permitting stable unit recording over several hours. Stimulating electrodes were positioned in ipsilateral cortical white matter guided by recent studies of the anatomical organization of direct corticostriate projections (Knowles et al 1980 Soc. Neurosci. Abst. 6:270).

Post-stimulus histograms (PSH) were obtained for nine striatal units recorded from five animals using cortical stimulation consisting of single pulses, 0.15 ms and 200 to 1000μA. PSHs showed robust evoked unit responses that consisted of excitation-inhibition sequences, in agreement with results of numerous acute studies. Responses requiring the least stimulus current were obtained with the recording electrode positioned in striatum 0.3 to 0.9 mm posterior to Bregma (level skull) and 3 to 4 mm lateral to midline, and the stimulating electrode placed in the forepaw area of primary somatosensory cortex, at Bregma and 4 mm lateral to midline.

During awake, motionless behavior, excitatory responses showed an average latency to onset of 6.6 ms and a duration of 31 ms, followed by an inhibitory period that averaged 90 ms. In contrast, during slow-paced treadmill walking, the excitatory peak in histograms for all nine units was significantly reduced by an average of 57%. This reduction in excitatory response was not due to a generalized suppression of striatal unit activity, since over 70% of striatal units in these studies showed increased firing rates during movement.

These results indicate that mechanisms exist which regulate neuronal transmission from cortex to striatum as a function of behavior. Further tests are required to explore the possibilities that these effects are 1) due to altered monosynaptic transmission in striatum, 2) due to changes in the number of corticostriatal fibers activated or 3) dependent upon activity in other afferent systems such as the nigrostriatal dopamine input.

Supported by NIAAA 3901, DA02338, and the Biological Humanics Foundation.

278.9 MOVEMENT RELATED NEURONS IN THE ENTOPEDUNCULAR NUCLEUS OF THE CAT
J.R. Morse and T.I. Lidsky (Sponsor: J. McLaughlin), SUNY at Stony Brook, Stony Brook, N.Y. 11794.

As part of an ongoing series of experiments investigating the sensory-motor functions of the basal ganglia, the responses of 165 entopeduncular nucleus cells to somatosensory stimuli were sampled in behaving cats. In most cases these neurons were also monitored during head movement. An acrylic plug containing the chronic recording chamber was fixed to the skull and also allowed the cat's head to be held painlessly in a holder which could rotate left or right. Movements were controlled by the investigator (passive) or the cat (active). A potentiometer mounted in the head restraint provided a record of these movements.

Approximately 15% of the sampled neurons changed firing rates in relation to head movement. Though firing changes did not precisely precede or follow the onset of movement in most cases, responsive units displayed a repeatable increase or decrease in activity accompanying movement. A number of cells responding during active movements did change firing rates prior to movement onset. In some cases there was a distinct difference in the responses to left vs. right movements. Approximately 54% of these movement related cells also responded to somatosensory stimulation. Of these, 50% had facial fields, 43% had fields involving back, neck, or shoulders, and 7% were responsive to visual stimuli.

Both clinical and experimental data indicate that the basal ganglia play a role in head positioning and oro-facial movement. The entopeduncular nucleus like other components of the basal ganglia processes sensory information from the face as well as information related to movement of the head. This kind of information could be important in the control of behavior relevant to prey capture and ingestion.
(Supported by NINCDS Grant NS16054).

278.10 ACTIVITY OF NEURONS IN THE GLOBUS PALLIDUS IN RELATION TO DIRECTION OF MOVEMENT OR PATTERN OF MUSCULAR ACTIVITY.
S.J. Mitchell, R.T. Richardson, F.H. Baker, and M.R. DeLong. Depts. of Neurology and Neuroscience, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

The direction of limb movement has been shown to be an important determinant of neuronal discharge in the basal ganglia. In a recent study neural activity in the putamen (the receptive portion of the basal ganglia) was most often related to the direction of movement rather than to the pattern of muscular activity. The present study was undertaken in order to determine whether neural activity in the globus pallidus (GP), which receives input from the putamen and in turn gives rise to output from the basal ganglia, is also preferentially related to the direction of movement rather than the pattern of muscle activity.

Macaque monkeys were trained to perform an arm visuomotor tracking task similar to that employed in the earlier putamen study. Animals made flexion or extension movements of the elbow. After aligning the arm within a central window and holding for a variable period, a sustained 150g flexor or extensor load or a brief 75g perturbation in the flexor or extensor direction was applied to the arm via a torque motor. The animals had to reposition their arm within the center window, hold for a variable period, and then, in response to a target movement, make a rapid elbow flexion or extension movement with either an assisting or opposing load (or no load). The animals then held at the target for a variable period which was followed by a liquid reward. Neurons were studied in both the external (GPe) and internal (GPi) segments of the GP and in the ventral GP (GPv) which lies below the anterior commissure.

Of the movement related neurons in GP, 39%, 53%, and 13% in GPe, GPi, and GPv respectively were related to the direction of arm movement. None of the cells studied showed a pattern of activity similar to that of the muscles. Of the neurons responding to load application, 49%, 83%, and 46% in GPe, GPi, and GPv respectively showed directional responses. Also, 45%, 20%, and 22% of cells in GPe, Gpi, and GPv showed a relation to static load.

These findings indicate that neurons in the GP, as in the putamen, are related to the direction of movement irrespective of the pattern of muscular activity used to make the movement. This, together with the finding of earlier studies that GP neurons are related to the amplitude/velocity of movement indicates that the basal ganglia may play a role in the control of movement parameters (i.e. the direction, amplitude, and velocity of movement) rather than in the selection or control of specific muscles.

278.11 ACTIVITY OF NEURONS IN THE MACAQUE NUCLEUS BASALIS OF MEYNERT IN A VISUOMOTOR TRACKING TASK.
R. T. Richardson, S. J. Mitchell, F. H. Baker, and M. R. DeLong. Depts. of Neurology and Neuroscience, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

Some neurons of the nucleus basalis of Meynert (nbM) have been shown to respond phasically with reaching movements and with juice delivery. To better characterize these correlations, nbM neurons were studied in macaques trained to perform a visuomotor tracking task for a juice reward.

During the task, the arm was restrained to allow only flexion or extension of the elbow. The animal first aligned his arm with a central target light and held it there for a variable time. A brief perturbation or a sustained load in either the flexor or extensor direction was applied to the arm via a torque motor. The animal had to recover his original position and hold again for a variable time before the target light moved either to the left or the right. The monkey made a rapid flexion or extension with assisting or opposing loads (or unloaded) in order to realign his arm with the target light. After a third variable hold time, juice was delivered to the monkey and simultaneously the lights were extinguished and the load removed.

Many neurons in the nbM proper (i.e. in the subpallidal region of the basal forebrain) and nbM border neurons (those nbM cells located in the medullary laminae of the pallidum and at the edges of the anterior commissure and the internal capsule) were active when the load was applied and/or during the movement segment of the task. Very few of the responding nbM proper neurons showed a directional preference; however, nbM border neurons did show a directional preference about as frequently as did pallidal neurons. In contrast to pallidal cells, only a few neurons in each nbM area had a tonic response to the sustained load. No nbM neuron had a dynamic load-related response during the movement segment. These results suggest that nbM neurons do not encode information about specific parameters of movement as do pallidal neurons, but rather that they encode some general feature about movement or its occurrence. Also, nbM border neurons seem to have properties more similar to pallidal neurons than do the nbM proper neurons.

Twice as many neurons in the nbM proper as in the pallidum or the nbM border areas responded to delivery of juice. The neurons which did respond to juice delivery frequently also responded to all movements. This suggests that these neurons may encode major events in the animal's behavior or environment rather than reward or drinking per se. Some nbM neurons showed stimulus-locked responses to target onset or offset and/or to light movement, indicating a possible response to visual input.

278.12 NEURAL CORRELATES OF ISOMETRIC FORCE IN "MOTOR" THALAMUS AND GLOBUS PALLIDUS.
M.-C. Hepp-Reymond, R. E. C. Anner-Baratti* and J. H. J. Allum. Brain Research Institute, Zurich University CH-8029 Zurich.

There is now convincing evidence that precentral cortical neurons are tightly involved in the control of fine-graded isometric contractions of finger muscles. The possible participation of the "motor" thalamus and the globus pallidus (GP) in this control was investigated in two alert monkeys trained to generate, between thumb and fore-finger, two successive step-and-hold increases in force, the first being lower (0 to 0.2 N) than the second (ca. 0.2 to 0.7 N). Neurons were recorded according to standard techniques and low current stimulation (5 - 30 µA) was applied through the recording microelectrode.

Following histological reconstruction, 115 neurons with stable relation to the motor task were retained for analysis. From those 55 were located in the "motor" thalamus (VL/VLo nuclei) and 60 within the GP (44 in GPi and 16 in GPe). The discharge patterns were classified into two basic types: One population which was rarely encountered in the finger region of Area 4 was called atypical and displayed sequences of phasic and tonic firing rate modulations without a simple relation to the force traces. The other neuronal group, called typical, had discharge patterns similar to those in Area 4, and represented 58% of the VL/VLo and GP neurons.

A large group of typical and atypical neurons (71% of the VLo/VPLo and 88% of the GP neurons) showed modulations of their tonic firing rate with static force. For the typical neurons monotonic increase of the firing frequency over two or more force levels was observed in 72% of the thalamic and only in 46% GP neurons. Mean rate-force slopes were 63 Hz/N for the thalamic and 62 Hz/N for the GP neurons, being similar to the cortical slopes (Hepp-Reymond and Diener, Exp. Brain Res., Suppl. 7, 1983). Among the neurons whose activity decreased with force only a small percentage in both subcortical regions showed a monotonic decrement. The onset of change in firing rate measured at the time of force increase from the low to the high level occurred later in the GP than in the thalamic or cortical neurons.

These data strongly suggest that neuronal subpopulations in the thalamus and the GP participate in the control of fine-graded force exerted by the fingers in a way similar to Area 4, the similarity being more evident for the thalamic than for the GP neurons.

Supported by Swiss National Fond 3.585.79 and 3.616.8, and by the Dr. Eric Slack-Gyr Foundation.

278.13 EFFECTS OF APOMORPHINE IN SUBSTANTIA NIGRA PARS RETICULATA: VARIABLE RESPONSES IN NORMAL RATS AND INHIBITORY EFFECTS IN 6-HYDROXYDOPAMINE LESIONED RATS. E.K. Lee*, B.L. Waszczak, and J.R. Walters (SPON: D.A. Bergstrom). NINCDS, NIH, Bethesda, MD 20205.

The substantia nigra (SN) pars reticulata receives a major striatal input and transmits information to premotor nuclei outside the basal ganglia, including the ventromedial (VM) thalamus and superior colliculus (SC). Since neuronal activity in the pars reticulata can be considered to reflect net changes in striatal output function, it was of interest to determine how these neurons would respond to stimulation of striatal postsynaptic dopamine (DA) receptors. Previous studies in this laboratory revealed that, when given to awake, paralyzed rats, a single 320 µg/kg i.v. dose of the DA agonist apomorphine (APO) significantly increased the firing of neurons in the globus pallidus, a second nucleus which receives a major striatal input.

Extracellular, single unit responses of SN pars reticulata neurons to a 320 µg/kg i.v. dose of APO recorded in chloral hydrate anesthetized rats were extremely variable (n=29). To rule out concern that anesthesia was responsible for the variable responses, all further studies were carried out in paralyzed, locally anesthetized rats. However, reticulata responses to APO remained highly variable: 35% of cells excited by >25%; 24% inhibited by >25%; 41% of cells unaffected (± 25%) 5-10 min after injection (n=17). The average response for all cells tested was a moderate increase in firing by 39 ± 25%. Further studies were carried out on neurons which were identified as nigrothalamic or nigrotectal cells on the basis of antidromic activation from the VM thalamus or SC, respectively. Among nigrothalamic neurons, responses to 320 µg/kg APO were again highly variable: 32% of cells excited; 32% inhibited; 36% unchanged 5-10 min after APO. The average effect for all cells was an increase by 16 ± 5% (n=22). Nigrotectal cells tended to be either unaffected (57% of cells) or excited (36% of cells); only one cell was inhibited by >25%. The average response of nigrotectal neurons was an increase by 21 ± 11% (n=14).

In contrast to the variable responses observed in normal rats, APO caused a more consistent and often total inhibition of firing of both nigrothalamic and nigrotectal neurons in rats which received 6-hydroxydopamine (6-OHDA) lesions of the SN DA pathway 6-8 weeks previously. Of 17 nigrothalamic cells tested, 14 (82%) were inhibited by an average of 93 ± 19% of their baseline rates; only 2 cells were excited and 1 was unaffected. Similarly, of 21 nigrotectal cells recorded in lesioned rats, 17 (81%) were inhibited by an average of 82 ± 6% of their baseline rates; only 2 cells were excited and 2 were unaffected.

The variable response of pars reticulata neurons to i.v. APO in normal rats contrasts strikingly with the consistent excitatory responses to APO seen in the globus pallidus and suggests that complex or multiple indirect effects of the drug may contribute to the varied reticulata responses. However, in 6-OHDA lesioned rats, the existence of striatal DA receptor supersensitivity may specifically exaggerate an inhibitory DA-mediated striatal influence upon these neurons.

278.14 STUDY OF FIRING PATTERN OF NIGRAL DOPAMINERGIC NEURONS AFTER NEOSTRIATAL LESION IN RATS, EFFECT OF MUSCIMOL. D. Doudet*, C. Gross*, and B. Bioulac. Groupe Motricité, Lab. de Neurophysiologie, Univ. de Bordeaux II, 146 rue Léo Saignat, 33076 BORDEAUX CEDEX (FRANCE).

As shown by post-mortem analysis the major neuropathological trait of the Huntington's chorea is a degeneration of the intrinsic neurons of the neostriatum. The destruction of the neostriatum by kainic acid mimics that situation (Coyle, J.T., Biol. Psychiatry, 14 : 251, 1979). Nigral dopaminergic (DA) neurons in normal rats exhibit a regular pattern of discharge (Wilson, C., et al., Brain Res., 136 : 243, 1977). Their autocorrelogram show a pronounced rhytmicity. The neostriatal lesion induces, in most nigral DA-cells, the appearance of an electrophysiological syndrome characterized by a disorganized activity and a decrease in frequency (Doudet, D. et al., Neurosc. Letters, Suppl. 10, S154, 1982). We have examined in the present work the firing pattern of identified DA-neurons located in the pars compacta of the substantia nigra before and after a kainic acid neostriatal lesion. We have, then, tested the effect of muscimol, a GABAergic agonist, on this electrophysiological syndrome. *Methods* : Nigral DA-cells, which were recorded in normal and lesioned rats, were identified during recording period on the basis of location, electrophysiological and pharmacological characteristics. The baseline unit activity was recorded for at least 200 sec before muscimol injection. The drug has been intravenously administered (2.5 mg/kg). *Results* : In normal animals, muscimol was tested on 18 identified DA-neurons. All these neurons had a very regular autocorrelogram. Their mean frequency was low ($\bar{F}$: 3.60 spikes/sec). As previously described (Waszcak, B., et al., Brain Res. 188 : 135, 1980), the injection of muscimol induced an increase in frequency ($\bar{F}$: 5.46 spikes/sec), but did not alter the rhytmicity. In lesioned animals, muscimol was tested on 27 DA-neurons presenting the so-called electrophysiological syndrome. All these neurons had a very low frequency ($\bar{F}$: 2.62 spikes/sec) and an autocorrelogram without periodic fluctuations. Muscimol produced on 24 of these 27 neurons a frequency increase ($\bar{F}$: 4.07 spikes/sec). However, the main observation resides in the fact that 21 of these 24 DA-cells exhibited a reorganisation of their patterns of discharge. This reorganisation lasted over a mean period of 27 min. following the injection.

Conclusions : The abnormal activity of numerous nigral DA-cells, after neostriatal lesion, may finally impinge upon motoneurons via ascending and descending pathways and participate in the genesis of choreic movements. The present data suggest that : (1) the absence of GABAergic neostriato-nigral pathway may partly account for the DA-cell dysfunction ; (2) the GABA agonist could be of interest in choreic syndrome therapy.

(supported by C.N.R.S. E.R.A. 493).

278.15 GLUTAMATE'S ROLE IN THE SUBSTANTIA NIGRA (SN): EFFECTS OF (-)-BACLOFEN AND LESION OF THE CORTICONIGRAL PATHWAY ON ACTIVITY OF SN PARS COMPACTA AND PARS RETICULATA NEURONS. T.H. Lanthorn, M. Knight, L. Steardo* and J.R. Walters. NINCDS, Bethesda, MD 20205.

Several lines of evidence suggest there may be a glutamatergic pathway from prefrontal cortex to the substantia nigra (SN). This study initiates an examination of the influence of this pathway and glutamate on SN activity.

Single unit recordings of SN cell firing rates were made in chloral hydrate anesthetized rats to test the effects of systemically applied (-)-baclofen, a drug which blocks transmission at several glutamatergic synapses. (-)-Baclofen has been shown to slow dopamine (DA) cell activity (Grace & Bunney, Br Res Bull 5,S2:537, 1980). We confirmed this and found that pars reticulata neurons appear 2½ times less sensitive than DA neurons to the rate-reducing effect of (-)-baclofen. While 56% of the pars reticulata neurons (n=9) showed enhanced firing rates at lower doses of this drug, most (89%) were inhibited by increasing doses. The ED50 was 11.5 ± 3.7 mg/kg, iv.

To explore the role of a glutamatergic innervation of the SN from prefrontal cortex, unilateral transection of the frontal cortex was made in rats by a combination of knife cuts and aspirative lesion. Nine days later, glutamate content was determined in punches of SN by HPLC. The SN ipsilateral to the lesion contained significantly less glutamate than did the contralateral SN (37.6 ± 2.2 vs 57.2 ± 2.2 nmol/mg protein, n=5), supporting the idea of a glutamatergic projection from prefrontal cortex to the SN. Serine content was not significantly changed. Similarly lesioned rats were anesthetized with chloral hydrate for single unit recording studies 2-3 weeks after the lesion. All spontaneously active DA and all non-DA (reticulata) neurons for which a positive-negative spike could be found were recorded in 9 passes through the SN. DA cell activity was not significantly altered by the lesion; 0.89 ± 0.08 DA cells/pass fired 3.4 ± 0.33 spikes/sec in control rats (n=6), 0.77 ± 0.11 DA cells/pass fired 4.0 ± 0.40 spikes/sec in lesioned rats (n=15). Firing rate of reticulata cells was also unchanged; 18.2 ± 1.11 spikes/sec in controls and 16.1 ± 1.20 spikes/sec in lesioned rats. However, 1.62 ± 0.11 reticulata cells/pass were found in controls, while significantly fewer, 0.85 ± 0.14 cells/pass, ($p < .005$) were found in rats with frontal cortex lesions. Thus, while reticulata cells were less affected by (-)-baclofen than were DA cells, only in the reticulata was there a reduction in the number of spontaneously active cells after frontal cortex lesion. This change in the pars reticulata may result from the loss of glutamatergic input from prefrontal cortex to the SN or be mediated indirectly via effects of the lesion on other areas, e.g. striatum. The lack of effect of the lesion on DA cell activity suggests that DA cells are not tonically excited by corticonigral innervation.

278.16 THE BASAL GANGLIA AND THE CONTROL OF URINARY BLADDER MOTILITY IN THE RAT. A. Dray and R. Metsch*. Department of Pharmacology, University of Arizona, Medical School, Tucson, Arizona 85724.

The involvement of the central nervous system in the control of urinary bladder motility is poorly understood although it is apparent that both neurological lesions of the CNS and central drug action may produce bladder dysfunction. Presently, the role of the basal ganglia has been explored since both lesions (in Parkinson's disease) and stimulation (in cats) of the structures alter bladder motility. Experiments were made in adult rats (200-220 g) anesthetized with ketamine or urethane. Cystometry was performed routinely following cannulation of the bladder via the urethra. The bladder was filled in 0.1 ml increments until micturition contractions were initiated (overall 27-60 mm Hg). Micturition waves were constant for an individual animal as were the frequency of contractions. All manipulations in the brain were made using stereotaxic methods. Bipolar electrical stimulation (0.5-8 volts; 1-100 Hz; for 20 msec to 2 min) of the striatum inhibited micturition contractions. Repeated pulses of higher frequency were more effective than single shocks delivered at lower frequency. Bilateral stimulation was more effective than unilateral stimulation. Intrastriatal microinjections of dopamine (1-4 µg) or apomorphine (1-5 µg) either inhibited or stimulated micturition waves. Carbachol (1-5 µg) consistently reduced contraction frequency. These effects were reversed by haloperidol (1 µg) and atropine (1 µg), respectively. Chronic (14-20 day) lesions of the striatum made electrolytically or by kainic acid injections consistently produced "hyperactive" detrusor function revealed by cystometry, whereas 6-hydroxydopamine lesions of the substantia nigra produced a "hypoactive" bladder. These data suggest that the basal ganglia have a complex role in regulating urinary bladder motility and that the rat may be useful in evaluating the physiological and neurochemical basis for this regulation.

279.1 CORTICAL AND SUBCORTICAL EFFERENT PROJECTIONS OF THE MEDIAL GENICULATE NUCLEUS IN RAT. J.E. LeDoux, A. Sakaguchi, D.J. Reis. Lab of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

We examined the efferent projections of the medial geniculate neucleus (MG) in rat, a species in which such projections have not been analyzed using axonal transport techniques.

Wheatgerm agglutinin-conjugated HRP (30-40nl) was microinjected into MG in male, Sprague-Dawley rats (n=8). The diffusion sphere of the injection typically included the principal and magnocellular divisions of MG, with the dorsal division and adjacent regions such as the suprageniculate and peripeduncular nuclei inconsistently involved. In all cases labeled cell bodies were seen in the ipsilateral inferior colliculus and nucleus of the lateral lemniscus, thus confirming the placement of the injection in MG.

Each injection resulted in the anterograde transport of HRP to the ipsilateral neocortex, where two terminal zones were observed. The posterior zone overlapped with the area identified by Krieg (J.Comp.Neurol. 84, 221-275, 1946) as the primary auditory cortex (area 41), but also included most of areas 39, 40, and 2a. The smaller anterior projection zone was located in the anteroventral portion of the somatosensory cortex (area 2). In both zones, terminals were densest in cortical lamina III and IV, but terminals were also observed amongst densely packed cell bodies in the deep lamina.

Subcortically, terminals were observed in the ventromedial hypothalamus (VMH), central and lateral amygdala (AMY), and the ventral aspects of the caudate-putamen (C-P). Fibers giving rise to these terminals could be traced from the internal capsule. In each area, a few retrogradely labeled cell bodies could be identified amongst the terminals.

These findings identify the neocortical distribution of MG efferents and in addition demonstrate that MG projects to several subcortical areas involved in autonomic and somatomotor control. These projections could conceivably play a role in the expression of autonomic and behavioral responses elicited by acoustic stimuli (see Sakaguchi, LeDoux, and Reis, Neurosci. Abs., 1983). Supported by PHS grant HL 18974.

279.2 NEURONS OF LAYER III IN CAT PRIMARY AUDITORY CORTEX (AI): VARIETIES OF PYRAMIDAL AND NON-PYRAMIDAL CELLS. Jeffery A. Winer. Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

The structure of layer III cells and axons was studied in Golgi, Nissl, fiber, and electron microscopic preparations in adult cats. Layer III lies from about 400 to 800 µm beneath the pial surface, and is distinct from layer II by its somewhat larger neurons and the vertical orientation of their dendrites and axons, the denser plexus of horizontally and vertically arranged fibers, and the participation of many layer III neurons in the commissural system interconnecting the primary auditory cortices (Code and Winer, 1983, this volume). The layer IV boundary is marked by large, deep-lying layer III pyramidal cells whose basilar dendritic arbors often encroach into layer IV, and whose axons pass laterally in AI for considerable distances. Besides the commissural component, certain layer III pyramidal cells project to the ipsilateral AII (Winguth and Winer, 1983, this volume). There are five types of layer III pyramidal cells. In all, eleven varieties of neurons are distinguished on dendritic, somatic, and axonal morphology. Layer IIIa is dominated by small and medium-sized pyramidal cells whose apical dendrites rarely enter the upper part of layer I; their axon divides in layer III, often passing laterally. Larger, layer IIIb pyramidal cell dendrites may extend to layer I, their apical dendrites forming prominent vertical arrays, and their axons may descend while emitting extensive branches. Two kinds of stellate pyramidal cells--smooth or sparsely spinous, also occur. Non-pyramidal cells lie throughout layer III. Their round, oval, or fusiform somata, limited dendritic fields, and slender local axonal branches distinguish them from pyramidal cells, and their collaterals may contribute to the fine local axonal plexus in electron micrographs of the neuropil (Larue and Winer, 1983, this volume). The six kinds of non-pyramidal neurons include tufted or bitufted cells with vertically polarized dendrites; sparsely spinous stellate neurons with more spherical dendritic fields; stellate cells with sparse, poorly radiating dendritic patterns; bipolar neurons with a few sparse dendritic branches; multipolar cells with spherical, radiating dendritic arbors; and tiny neurons with slender dendrites and limited dendritic domains. Non-pyramidal cell axons ramify locally, often forming vertical arcades in layers II, III, and IV. The distribution of pyramidal and non-pyramidal cell axons suggests at least five targets for layer III neurons: local projections restricted to the cell's immediate vicinity; vertical branches to superior and inferior cortical layers, but limited to the approximate width of the cell's dendritic fields; longer, horizontal pathways chiefly terminating in AI; ipsilateral cortico-cortical projections, e.g., to AII; and commissural pathways. The manifold forms of layer III cells and their diverse targets in the auditory cortex imply that they participate in different pathways. This research was supported by USPHS grant R01 NS16832.

279.3 LAYER III OF CAT PRIMARY AUDITORY CORTEX (AI): AN ULTRASTRUCTURAL STUDY. David T. Larue* and Jeffery A. Winer. (SPONSOR: T. Nakada). Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

We studied layer III somata and neuropil as part of an inquiry into the organization of AI. Neurons were classified in the light microscope as either pyramidal or non-pyramidal on the bases of size, dendritic branching pattern and appendages, and axonal morphology from Golgi, Nissl, and plastic-embedded, toluidine blue-stained sections (Winer, 1983, this volume). In the electron microscope pyramidal cells typically have large, triangular somata with a thick dendrite emerging from the apical and thinner dendrites from the basal poles, prominent Golgi apparatus at the origins of dendrites, a nucleus with a single modest invagination, a rather patchy, darkly colored and abundant cytoplasm, and a ventrally directed axon. Non-pyramidal cells, in contrast, usually have smaller, round somata with smooth contours, an axon and thin dendrites arising without polarity, modestly developed Golgi apparatus, an eccentric nucleus with multiple, lobular invaginations, and a sparse rim of uniform and granular perinuclear cytoplasm. In electron micrographs pyramidal cell somata are about 25% larger than non-pyramidal perikarya (see Table below). Both cell types receive an average of 4.5 synaptic endings in mid-nucleolar thin sections. However, the morphology of axo-somatic endings differed markedly: 80% of those on pyramidal cell somata have flattened synaptic vesicles, while non-pyramidal cells receive almost equal numbers of endings with flattened or round vesicles. The pattern of axo-spinous and axo-dendritic synapes in the neuropil differed markedly from that of axo-somatic endings. Axon terminals with round vesicles comprise 88% of these endings, while the remaining 12% have flattened vesicles. Dense cored vesicles occasionally appeared in somatic endings and in the neuropil but those on somata were found only in flattened vesicle terminals; those in the neuropil were usually found in round vesicle endings. This implies that the patterns of synaptic inputs are specific to different kinds of layer III cells, and that the innervation of the neuropil is different. Since several types of layer III pyramidal and non-pyramidal cells project commissurally in AI (Code and Winer, 1983, this volume), or have a role in the cortico-cortical pathways connecting subdivisions of auditory cortex (Winguth and Winer, 1983, this volume), different patterns of synaptic organization of pyramidal and non-pyramidal cells might have functional significance in the spatial segregation of auditory input to particular cells. We are now studying the origins of these axonal endings. This research was supported by USPHS grant R01 NS16832.

Summary of Axonal Endings in Layer III of Primary Auditory Cortex

	Pyramidal cells (N=13)	Non-pyramidal cells (N=9)	Neuropil
Mean somatic diameter (s.d.)	16.6 (2.2 µm)	12.8 (1.8 µm)	----
Number of synaptic endings	60	40	438
Number of endings with flattened vesicles	48 (80%)	19 (47.5%)	54 (12%)
Number of endings with round vesicles	12 (20%)	21 (52.5%)	384 (88%)

279.4 HETEROGENEOUS ORIGIN OF COMMISSURAL NEURONS IN CAT PRIMARY AUDITORY CORTEX (AI). Rebecca A. Code and Jeffery A. Winer. Department of Physiology-Anatomy, University of California, Berkeley, California 94720

The cells of origin of the commissural system in cat primary auditory cortex were studied as part of an inquiry into the organization of AI. After injection of 1-6 µl of horseradish peroxidase conjugated to wheat germ agglutinin into the convexity of the middle ectosylvian gyrus, the contralateral AI was cut into serial, 60 µm thick frozen or Vibratome sections and processed using the DAB, Hanker-Yates, or TMB chromogens after a 48-72 hour survival. Retrogradely labeled commissural cells were found only in layers III, V, and VI of AI. Of the 7164 labeled cells studied in 4 cats, 71% (5086) were found in layer III and 29% (2078) were found in layers V-VI. Labeled cells often form clusters, columns, or strips within layer III; between these clusters few or no cells were labeled. The distribution of peroxidase-labeled cells is topographically organized in the contralateral AI. If the injection was primarily in the rostral part of AI, the maximum number of labeled cells was in the rostral part of the contralateral AI. If the injection included all of AI, then most labeled cells lay throughout AI on the contralateral side. The control for the localization of the injection in AI was the pattern of retrograde labeling in the ipsilateral ventral nucleus of the medial geniculate body, which also varied systematically with the position of the injection in AI. The area of the retrogradely labeled somata was measured with an electronic digitizer from drawings made with a camera lucida. About 80% of the labeled cells in layer III are small, medium-sized or large neurons (average somatic area: 294.8 sq. µm; see Table below; Nissl counterstain). Their triangular somata and thick primary dendrites suggest they may be pyramidal cells. Approximately 20% were classified as non-pyramidal cells; their average somatic area is 216.9 sq. µm, and their oval or fusiform somata are typical of non-pyramidal cells (Winer, 1983, this volume). A t-test revealed a significant difference ($p<.001$) between the average area of labeled pyramidal and non-pyramidal somata in layer III. We believe that they probably represent two morphologically distinct inputs arising from layer III, in addition to the contribution of cells in layers V and VI. The correlation of the types of labeled pyramidal and non-pyramidal cells with the neuronal populations and patterns of synaptic endings in layer III is the subject of current study. This research was supported by USPHS grant R01 NS16832.

Horseradish Peroxidase-Labeled Commissural Cells in Layer III of AI

Type of cell	Average somatic area (sq. µm)	Standard deviation (sq. µm)	Range (sq. µm)	Number of cells
pyramidal	294.8	93.2	149.9-681.8	325
non-pyramidal	216.9	55.8	117.4-446.4	83
pyramidal and non-pyramidal	278.9	92.3	117.4-681.8	408

279.5 ARCHITECTURE OF LAYER II IN CAT PRIMARY AUDITORY CORTEX (AI) AND SOME IPSILATERAL CORTICO-CORTICAL CONNECTIONS. Sandra D. Winguth and Jeffery A. Winer. Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

As part of an inquiry into AI we studied the structure and connections of layer II in adult cats with Golgi, Nissl, and axoplasmic transport methods. Layer II lies between 200-400 μm deep to the pial surface and is distinct from the cell-poor layer I, and from the larger neurons, especially pyramidal, of layer III. In layer II small and medium-sized pyramidal cells occur, their apical dendrites branching extensively. Their basilar dendritic arbors are rarely as robust as those of layer III pyramidal neurons, nor are their somata as large or angular. Their axon projects within layers II and III and may have side branches. Smooth multipolar stellate cells have limited, primarily vertical dendritic fields radiating irregularly from an oblate somata. The axon has many (30 or more) collaterals, some locally distributed, others projecting laterally and ventrally, but most confined to layer II. Chandelier cells have multiangular somata with well developed apical and sparsely branched basilar dendrites. The latter may turn and ascend to layer I, often entering it, while the axon forms extensive side branches and projects laterally within layer II, and toward layer III. Moderately spinous or spiny stellate cells have flask-shaped perikarya and 2-5 primary dendrites. These ramify in layer II but the extremities, often encrusted with appendages, may invade adjacent layers, and their axons branch locally. Sparsely spinous stellate cells have round somata but, in contrast to the chandelier cells their dendrites are thinner and have a radiate branching pattern irregularly filling a sphere. Their axon has many local branches directed toward layer III and fewer lateral ones. Bipolar and bitufted cells have long vertical dendritic arbors, often running to layers I-III, and with variable numbers of dendritic appendages. Their drumstick-like somata lie throughout layer II. Other types of cells may also occur. Small horseradish peroxidase injections in AII retrogradely label ipsilateral AI somata in layers I-VI. Labeled layer II somata have various, usually triangular shapes, and may form small clusters or strips remote from the AI-AII border. The locus of retrogradely labeled cells in the medial geniculate body was a control for the cortical locus of injection. Since layer II neurons are not retrogradely labeled except by ipsilateral cortical injections, we conclude that their connections differ from those of layer III cells (Winer, 1983, this volume; Code and Winer, 1983, this volume; Larue and Winer, 1983, this volume), and layer V cells (Games and Winer, 1983, this volume), each of which participates in the commissural system. In this sense layer II is more closely allied in its organization with layer IV. This research was supported by USPHS grant ROl NS16832.

279.6 PROJECTIONS OF LAYER V IN AUDITORY CORTEX TO THE RAT INFERIOR COLLICULUS: CELLS OF ORIGIN AND NEURONAL ARCHITECTURE. Kate D. Games and Jeffery A. Winer. Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

We injected horseradish peroxidase into the inferior colliculus of adult rats to compare the retrogradely labeled layer V cells with neurons characterized in Golgi and Nissl preparations as part of a study of the organization of auditory cortex. Layer V lies between 700-875 μm deep to the pial surface and is distinct from the smaller, primarily non-pyramidal somata of layer IV, and the heterogeneous cells of layer VI. In Golgi preparations we commonly observed three varieties of layer V pyramidal cells. Many pyramidal cells have well developed basilar dendrites and a stout apical dendrite. Stellate pyramidal cells have more spherical dendritic fields and a pronounced radiate branching pattern. Inverted pyramidal cells are also present. Pyramidal cell somata are about 15-22 μm in diameter. Among the non-pyramidal cells are bipolar or bitufted neurons with fusiform somata and vertically polarized dendritic fields, and stellate cells with smaller, more radiate dendritic domains and oval or flask-shaped somata averaging 7-15 μm in diameter. Large horseradish peroxidase injections in the inferior colliculus labeled approximately half of the layer V cells in the auditory cortex above the rhinal sulcus. Many such cells have a triangular perikaryon and tend to be large. Smaller, fusiform somata were rarely labeled. In sections with many labeled neurons in layer V, groups of 5-10 labeled cells were interspersed among patches of unlabeled neurons, and single, labeled neurons were uncommon. Smaller horseradish peroxidase injections produced the same pattern of cortical retrograde transport, though on a lesser scale. The number and distribution of retrogradely labeled cells in the auditory cortex, contralateral inferior colliculus, and the subdivisions of the cochlear nucleus (and other nuclei of the auditory brain stem), were systematically related to the size and locus of the injection. In the cat some layer V auditory cortex cells project commissurally (Code and Winer, 1983, this volume) and ipsilaterally (Winguth and Winer, 1983, this volume). The possibility thus exists that layer V participates in three efferent pathways. This research was supported by USPHS grant ROl NS16832.

279.7 LAMINAR DISTRIBUTION AND DENDRITIC BRANCHING OF SPINE FREE NON-PYRAMIDAL NEURONS IN RABBIT AUDITORY CORTEX. N. T. McMullen, E. M. Glaser and M. Tagamets*. Dept. Physiology, Univ. of Maryland Sch. of Medicine, Baltimore, Maryland 21201.

A computer microscope study of the morphometry and laminar distribution of spine free nonpyramidal neurons in electrophysiologically verified primary auditory cortex was carried out in 3 adult rabbits. The laminar and tangential locations of all impregnated neurons in 300 um thick Golgi-Cox Nissl sections through primary auditory cortex were determined. These neurons were classified as pyramidal(P); spine free nonpyramidal(NP) and "other"(O, which included spiny, bipolar and small nonpyramidal cells). The three cortices were comparable in terms of density of impregnation(350 neurons/mm^3), relative proportion of cell types impregnated(87%, 9% and 4% for P, NP, and O respectively) and impregnation frequency(0.92%). The spine free NP variety constitutes 72% of all nonpyramidal cells present. They are found in a mid-cortical stratum approximately 800 um wide with peak concentrations at 450 to 750 um beneath the pia corresponding to lamina III and IV. A total of 60 spine free NP were studied with our image combining computer microscope (Glaser et al, J. Neurosci. Meth., '83) The average cell has a large soma(376 um^2), 7.9 dendrites and 44 branches. Total dendritic length averages 3087 um. 56% of the dendritic length and 52% of the branches are found in the 2nd and 3rd order segments. A combination of dendritic stick, Fourier and statistical analyses revealed a highly significant spatial orientation of their dendrites along a vertical(perpendicular to the pia) axis parallel to the apical dendrites of neighboring pyramids. NP cells in all three cortices also exhibited a significant tangential orientation of dendrites along a dorso-ventral axis. This appears to be normal to the isofrequency contours of rabbits mapped electrophysiologically. An examination of NP dendrite systems using a 3-D Sholl-type radial analysis showed that the pronounced vertical orientation is sculptured from an initially random distribution of dendrites as a result of: a) vertically directed dendritic growth and branching as dendrites grow centrifugally and b) decreased branching and rapid termination of dendrites growing tangentially. The longest dendrites are those directed toward the white matter. Supported by NIH Grants NS17861, GM27165 and a Bressler Research Award from the University of Maryland School of Medicine.

279.8 TERMINATION PATTERNS OF THALAMIC, CALLOSAL, AND ASSOCIATION AFFERENTS OF THE PRIMARY AUDITORY AREA IN THE RHESUS MONKEY. D. N. Pandya and D. L. Rosene, VA Medical Center Bedford, MA and Boston University School of Medicine.

Following radiolabelled amino acid injections of the medial geniculate body (MGB), supratemporal plane (STP) and superior temporal gyrus (STG) in the rhesus monkey, it is observed that the thalamic afferents to the primary auditory area were most prominent. These thalamic afferents were organized in the form of distinct bands, with most of the terminals being localized in layer IV with some extension of terminals in layer III. The callosal afferents by contrast were considerably less pronounced and occurred in spatially separated clusters, terminating in layer IV as well as layer III in the primary auditory area. The association afferents from the STG to the primary auditory area terminated in layer I in a continuous band.

In order to determine the extent to which callosal afferents overlap with the thalamic terminations, in one animal an isotope injection was placed in the MGB and the corpus callosum was sectioned. The thalamic terminations (labelled grains) and callosal afferents (fiber degeneration) were compared in adjacent sections prepared with the autoradiographic and silver impregnation (Fink-Heimer 1961) techniques respectively. The results showed both overlap and interdigitation patterns for these afferents within the primary auditory area.

Thus it seems that for the most part the thalamic, callosal and association inputs to the primary auditory area are conveyed in a topographic manner. There is, however, evidence for convergence of these inputs within the primary auditory area.

Supported by ENRM Veterans Hospital, MA by NIH Grant NS 16841 and NS 19416.

279.9 A GOLGI ANALYSIS OF THE AUDITORY CORTICES OF THE MONKEY. P. B. Cipolloni*, D.N. Pandya, and V.M. Knowlton,*(SPON: M.Malone) V.A.M.C., Bedford, MA and Boston University School of Medicine

The auditory cortical neuronal cell types of the monkey have been analyzed in adult rhesus monkeys using two Golgi impregnation techniques. (Braitenberg, V., Guglielmotti, V. and Sada, E., Stain Tech., 42: 277-282, 1967; Valverde, F., Contemporary Methods in Neuroanatomy, W.J.H. Nauta and S.O.E. Ebbesson (Eds). Springer-Verlag, New York,1970.)

The neuronal cell types can be divided into two classes based on the presence or absence of dendritic spines. The spinous neurons are segragated into typical pyramidal neurons,"star pyramids", spinous bipolar and spinous stellate neurons. The typical pyramidal neurons have their cell bodies in all cortical layers except layer I and vary in size. They all have a pyramidal or round cell body, and an apical dendrite coursing perpendicular to the cortex toward the pia, with varying numbers of tangential branches. The "star pyramid", usually found in layer IV, has a more symmetric dendritic array than the typical pyramidal neuron. At times one superficial dendrite may course into layer III. The cell bodies of spiny bipolars may be in layers III or V. They have a prominent apical shaft coursing directly toward the pia and an equally prominent basal shaft coursing either directly or obliquely toward the white matter. The axons of all the above neurons arise from the deeper portions of the somas and course to the white matter. Spinous stellate neurons are only occasionally seen and then only in layer IV. The dendrites of these cells have a symmetrical trajectory around the cell body. The axon of these cells may arise from the cell body or one of the dendrites.

The non-spinous neurons can be divided into stellate, bitufted, bipolar, and neuroglioform neurons. The smooth stellate neurons are found in layers II through VI, usually have spherical cell bodies and radially oriented dendrites appearing to occupy a spherical field of varying size. The bitufted cell has a spherical to elliptical cell body. Several dendrites arise from the superficial and deep poles of the soma and are arrayed in an hour-glass distribution. The smooth bipolar cell has a cigar-shaped cell body which is usually radially oriented in the cortex and has apical and basal main dendrites with each main dendrite having a few tangential branches. The axonal plexus of these three neuronal cell types vary markedly in terms of complexity. The neuroglioform neurons are observed in layers II, III,and V. These very small neurons have numerous short dendrites totally confined to one cortical layer with its axons tightly entwined within the dendrites.

Supported by E.N.R.M. Veterans Hospital, Bedford, MA and by NIH Grant N.S. 16841.

279.10 RESPONSES OF SINGLE NEURONS IN CAT PRIMARY AUDITORY CORTEX TO TONE- AND NOISE-BURST STIMULI. D. P. Phillips*, A. D. Musicant*, S. S. Orman*, G. F. Wilson* and C.-M. Huang* (SPON: R. M. Benjamin). Dept. Neurophysiol. and Waisman Ctr., Univ. Wisconsin, Madison WI 53706.

Most cat primary auditory cortex (AI) neurons are sharply tuned to a best frequency (BF), and their spike counts are either a monotonic or a nonmonotonic function of the intensity of a BF tone. In the present study, we gathered detailed data on the frequency-intensity response areas of AI cells, and have obtained evidence for an association between the selectivity of a neuron to tone frequency and intensity on the one hand, and its ability to respond to broad-band noise on the other. All data were obtained from single neurons in barbiturate anesthetized cats to which tone- and noise-burst stimuli were presented through calibrated, sealed stimulating systems.

In general, neurons which responded to noise bursts showed spike counts which were monotonic in shape for most or all tone frequencies to which the neurons were sensitive. The spectrum level of a neuron's BF in a threshold noise stimulus was almost always lower than its pure tone threshold, suggesting that at threshold levels, the neuron was responding to energy in the noise stimulus summed over a relatively wide bandwidth. For those neurons whose noise spike count functions were also monotonic, intensity dynamic ranges were typically wider for noise than for tones. The fact that other neurons with monotonic tone spike count functions displayed nonmonotonic noise spike count functions suggests that the high intensity parts of their tone response areas may have been bordered by inhibitory regions.

The majority of neurons failing to respond to noise stimuli were those whose pure tone spike count functions were strongly nonmonotonic such that their spike counts fell to zero at high stimulus levels, regardless of tone frequency. These neurons had completely circumscribed frequency-intensity response areas, responding to tones only within narrow domains of frequency and intensity. These cells were less common than monotonic neurons, and appeared to be segregated into small cortical loci approximately 300 microns in diameter. The nonmonotonic shape of these cells' pure tone spike count functions must be produced by inhibitory processes because the cochlear output shows no such property. The insensitivity of these cells to noise stimuli of any intensity suggests that their excitatory tone response areas may be flanked by side-band inhibition.

(FO5-TWO3102, BNS7912939, HDO3353, NS12732, NSO7026)

279.11 STABILITY OF INTENSITY FUNCTIONS OF SINGLE NEURONS IN AUDITORY CORTICAL FIELD A-I. T. McKenna, D.M. Diamond*, and N.M. Weinberger. Dept. of Psychobiology, Univ. of Calif. Irvine, Irvine, CA 92717.

The sensory evoked discharges of auditory cortical neurons are modified during associative learning. We have assessed the reliability of sensory encoding of single neurons in a nonlearning situation by obtaining intensity functions in unanesthetized animals, with control of peripheral factors. Stability of stimulus encoding was measured by repeatedly determining intensity functions of single auditory cortical neurons across time and different states of arousal, in chronically prepared cats under neuromuscular blockade to insure stimulus constancy at the periphery. EEG and pupillary dilation were monitered continuously. A series of tones of 100 to 300 ms duration, at best frequency, were delivered at 10 different intensities (25 trials per intensity). This series was then repeated as many as 4 times, at 15 min. intervals. For some series, no stimuli were given during the 15 min. wait, nor between blocks of trials. During some of these series the animals exhibited slow wave activity and pupillary constriction. In other series the animal was aroused by single paw shocks delivered either before a series, or prior to each block of 25 trials. Over 70% of the neurons sampled exhibited no significant changes in the intensity function (plot of means of discharge rate for interval of peak response minus prestimulus rate) under all conditions examined. Some neurons exhibited stable intensity functions although background discharge rates had decreased over series which were accompanied by an increase in the level of arousal. Intensity functions were of 3 types: a) monotonic b) nonmonotonic and c) multipeaked, irregular. Intensity functions for the first 2 types of cells were predominately stable, whereas those for the 3rd category were not.

These results contrast with previous studies of the influence of state of arousal on the responsiveness of auditory cortical neurons in behaving monkeys, which reported that many or most neurons were strongly modulated by behavioral state. However, factors such as middle ear muscle activity, species differences, auditory areas sampled, or contextual and task related variables need to be considered in assessing the influence of arousal on the neuronal encoding of stimulus dimensions such as intensity.

Supported by NS 16108 and a grant from Monsanto Corp.

279.12 FREQUENCY DISPARITY SELECTIVITY IN CAT PRIMARY AUDITORY CORTEX (AI). J.R. Mendelson and M.S. Cynader, Dept. of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada.

The intensity and timing relationships of acoustic stimuli in the two ears are important cues for sound localization. When a sound source moves from left to right the stimulus amplitude increases in the right ear and decreases in the left. Because the head attenuates high sound frequencies more strongly than low frequencies, high frequency components will be diminished relative to low frequencies in the left ear. The overall peak of the frequency spectrum will thus be shifted toward higher frequencies in the right ear and lower frequencies in the left. The change in the shape and the peak of the frequency spectrum in the two ears will be a progressive function of the horizontal eccentricity of the stimulus. Little attention has been devoted to the neurophysiological investigation of this binaural frequency disparity as a possible cue to sound localization.

We examined the responses of cells to tones of different frequencies (delivered via ear speakers) in AI of anaesthetised, paralyzed cats. The characteristic frequency (cf) of each cell was determined and the cell classified as showing binaural facilitation (EE) or binaural inhibition (EI). Then, while the cf was presented to the contralateral ear, a series of pure tones of varied frequency were simultaneously presented to the other ear. Stimulus intensity was the same for the two ears.

Of the EE cells studied above, about 1/4 showed the greatest binaural facilitation when there was no difference in the frequency of the tones presented to the two ears. In contrast, nearly 3/4 of these cells gave their greatest binaural response when tones with different frequencies were presented. Approximately 1/3 of the EI cells showed their deepest inhibition with binaural presentation of the same frequency. The remaining EI cells showed their deepest inhibition when different frequencies were presented to the two ears. In both the EE and EI cells that responded to non-zero frequency disparities between the two ears, about 50% showed the greatest degree of facilitation (or inhibition) when the ipsilateral ear received higher frequencies than the contralatral ear, while the other 50% responded best (or showed greatest inhibition) when the ipsilateral ear received lower frequencies than the contralateral ear.

The results show that the responses of both EE and EI cells in AI are strongly dependent on the relative frequency content of stimuli in the two ears. The frequency disparity cue studied here appears to play a potentially important role in controlling the responses of both cortical cells and the behaving organism to sound sources of different eccentricities.

279.13 LAMINAR ANALYSIS OF SINGLE UNIT ACTIVITY IN AUDITORY CORTICAL FIELD AII OF CAT: STABILITY OF TUNING FUNCTIONS. D.M. Diamond*, N.M. Weinberger, Dept. of Psychobiology, UC Irvine, Irvine, CA.

Previously, we reported that every cell recorded in AII displayed discharge plasticity during associative learning (Neurosci. Abst. 8:317,1982). This investigation is the first in a series of studies designed to characterize the functional significance of such plasticity. Specifically, changes in auditory cortical activity during learning may represent alterations in receptive field characteristics. Preliminary to studying the possible effects of learning on receptive fields, this report is concerned with the degree to which tuning curves are stable in AII.

Single unit activity was recorded in chronically prepared cats under neuromuscular blockade to insure stimulus constancy at the periphery. The responses of single cells to tones of various frequencies were determined initially and again after a period of 15 to 30 minutes of silence.

There are two major findings related to an overall description of AII unit activity and of the stability of sensory evoked responses. First, the breadth of tuning and background rate of activity change as a function of the depth from the surface. Cells from 200-800u have background firing rates of 5.0 sp/sec., exhibit complex evoked responses consisting of periods of inhibition and excitation and can have narrow tuning functions. Cells from 800-1200u have high background rates (14.4 sp/sec), exhibit homogeneous evoked responses consisting of excitation at a latency of 12-18 msec and are very broadly tuned. Cells located from 1200-2100u have low background rates (1.9 sp/sec) and rarely respond to tonal stimuli. However, they usually discharge to complex sounds such as keys jangling. Previous reports have described only broadly tuned cells in AII, but this may be due to the fact that the studies used anesthetics which suppressed the activity of the upper and lower layers. The broadly tuned cells which are located from 800-1200u are likely to be in layer IV and receive direct thalamic input, thereby making them less susceptible to anesthetic agents.

The second finding is that the tuning functions of AII cells in each respective layer are largely stable. Moreover, components of evoked responses such as onsets, offsets and periods of inhibition are highly reproducible over time. Hence, the frequency response characterisitics of AII cells are relatively invariant in the absence of training.

Supported by NS 16108 and a grant from Monsanto

279.14 EVIDENCE FOR CONNECTIONS BETWEEN AUDITORY CORTEX AND FRONTAL CORTEX OF THE BAT, PTERONOTUS PARNELLII. J.B. Kobler*, S.F. Isbey and J.H. Casseday. Neurobiology Program, U. of No. Carolina, Chapel Hill, N.C., 27514, and Depts. of Psychol. and Surgery (Otolaryngology), Duke Univ., Durham, N.C. 27710.

We present new anatomical evidence that reciprocal connections exist between auditory and frontal cortices and that cells in the frontal cortex respond readily to ultrasonic stimuli in this echolocating bat. We first noted prominent anterograde and retrograde labeling in dorsolateral frontal cortex after a large injection in auditory cortex of wheat germ-agglutinin conjugated to horseradish peroxidase (HRP). Further evidence that this frontal area is connected to the auditory pathway was obtained from HRP injections placed in physiologically defined parts of auditory cortex. These cortical areas, which respond to auditory stimuli and which receive input from the medial geniculate body, have reciprocal connections with frontal cortex. Electrophysiological methods were used in four experiments to place HRP injections in the area of frontal cortex that responds to acoustic stimuli. In all of these cases we found a similar pattern: cells were labeled in dorsal and anterior parts of the auditory cortex, and few labeled cells were found in the central part of the auditory field. This central part probably corresponds to primary auditory cortex, an area we defined previously by cytoarchitecture and the anterograde transport of ^{3}H-leucine from the ventral division of the medial geniculate body. Labeled cells were also found in the depths of the lateral sulcus, in cingulate cortex, and in areas dorsal and posterior to auditory cortex. The main thalamic input to the frontal area is from the mediodorsal nucleus. In addition, the frontal area receives a projection from cells in the suprageniculate nucleus, an area which we have found to project to the auditory cortical field.

Single units and evoked responses to tone pips were mapped with microelectrodes in the frontal region in bats lightly anesthetized with metofane. Most units responded broadly to frequencies between 10 and 120 kHz and often showed increased sensitivity and sharp tuning to frequencies around 60 kHz, which is the most intense harmonic of this bat's sonar pulse. Latencies ranged from as short as 10 msec to greater than 75 msec. Units habituated readily and frequently responded to tone pips with bursts of 2-8 spikes.

These observations that the frontal cortex of Pteronotus has well developed connections with the auditory system is significant in view of this animal's sophisticated biosonar behavior. The results are particularly intriguing in view of current hypotheses implicating frontal cortex in functions such as spatial orientation and vocalization. [Supported by NSF grant BNS 82-17357.]

279.15 THE EFFECTS OF AUDITORY CORTICAL LESIONS ON SEVEN CHOICE SOUND LOCALIZATIONS BY FERRETS. Gerard Kavanagh* and Jack B. Kelly. (SPON: E.W. Peterson) Department of Psychology, Carleton University, Ottawa, Ontario, Canada, K1S 5B6.

Auditory cortical lesions have failed to produce marked impairments in sound localization in rats tested under various stimulus conditions in either two or seven choice sound localization. In contrast, a number of reports have indicated that severe deficits occur in other mammalian species following equivalent lesions. The possibility has been suggested that differences exist among mammals in the degree of cortical dependency of sound localization. For purposes of further comparison, we have undertaken studies of the effects of auditory cortical lesions using ferrets as subjects. Ferrets were selected as a readily available laboratory species with a well developed neocortex and a likely compatibility with behavioral training and testing procedures already established for rats. Results have shown the ferret to be an excellent species for behavioral studies. Normal animals rapidly learn to execute spatial responses in a semicircular maze for water reinforcement. They are capable of localizing sounds in either a two choice or a seven choice test situation and exhibit highly reliable levels of performance. The effects of cortical lesions have been examined in two cases following bilateral lesions of auditory cortex. Lesions included both primary auditory cortex (AI) and surrounding areas receiving projections from the medial geniculate body. Both animals had severe postoperative impairments in seven choice sound localization even when the stimulus, a train of clicks repeated at two per second, was left on until a response was completed. Neither animal was capable of performance above the level expected by chance when tested with a single click presented at the beginning of each trial. A third animal, tested following unilateral ablation of auditory cortex was found to have reduced performance contralateral to the side of the lesion. Impairments were evident with both two per second and single clicks. Deficits persisted with repeated testing over a prolonged postoperative recovery period. We conclude that ferrets are severely impaired in the ability to localize sounds in space following lesions of auditory cortex.

(This research was supported by NSERC grant, 7654.)

279.16 THE EFFECTS OF AUDITORY CORTICAL LESIONS ON SEVEN CHOICE SOUND LOCALIZATION BY THE RAT. P.W. Judge* and J.B. Kelly. Dept. Psychology, Carleton University, Ottawa, Ontario, Canada, K1S 5B6

Previous studies of the effects of auditory cortical lesions in the rat have shown only minor impairments in two-choice sound localization following large bilateral lesions (Kelly, J. Neurophysiol., 44, 1161-1174, 1980). The following study was undertaken with the expectation that deficits would be more readily apparent in a localization task which required responses to multiple sound sources. Albino rats were trained to localize sounds in a seven-choice test apparatus. The effects of both bilateral and unilateral lesions were evaluated. The test procedure required that an animal approach clicks delivered from one of seven loudspeakers located at 30 degree intervals around the perimeter of a semicircular maze. A click was presented when a subject made contact with a spout located in the center of the maze equidistant from the seven loudspeakers. This ensured that an animal's head was aligned with the 0 degree azimuth speaker position at the beginning of each trial. Animals were first trained to localize clicks repeated at two per second, and were then tested with a single brief click presented at the onset of each trial. Normal rats were capable of high levels of performance with two per second click trains, but attained only intermediate levels of performance with single clicks. Following unilateral lesions of auditory cortex, performance levels remained essentially unchanged. Comparison of animals with left versus right hemisphere lesions revealed no evidence of contralateral impairments in sound localization. Bilateral lesions resulted in slight impairments in localization although some ability to localize remained even with complete bilateral destruction of auditory cortex. These results are consistent with previous reports that lesions of auditory cortex in the rat do not produce severe impairments in sound localization.

(supported by NSERC grant 7654)

279.17 CONTRALATERAL DEFICITS IN HUMAN AUDITORY PERCEPTION AFTER UNILATERAL CEREBRAL LESIONS. T. Allard* and T.A. Zeffiro. Dept. Psychol., Mass. Inst. Tech., Cambridge, MA 02139 and Neurol. Serv., Mass. Gen. Hosp., Boston, MA 02114

Patients with unilateral cerebral lesions show subtle changes in the auditory perception of material presented to the contralateral ear. These changes have been described as defects in the perception of degraded speech sounds. This impairment is dissociable from disorders of language and could involve processing of nonverbal auditory stimuli. We present preliminary evidence that lesions of either hemisphere disrupt the perception of transients in both speech and nonspeech sounds.

Monaural detection thresholds for 1-ms and 500-ms bursts of white noise were obtained from 13 patients, aged 22 to 57, with left-hemisphere or right-hemisphere lesions. An adaptive $\hat{}$ estimation procedure was used in a computer-controlled, 2-interval, 2-alternative forced-choice paradigm with feedback. All subjects had normal and symmetrical thresholds for the 500-ms bursts. The normal group exhibited a symmetrical, 20-dB elevation in threshold for 1-ms bursts. In contrast, both patient groups showed a significantly greater contralateral increase in 1-ms thresholds when compared to performance with the ipsilateral ear ($p < .01$, matched-pair t-test).

This nonverbal perceptual impairment was associated with a deficit in the perception of a synthetic speech continuum varying voice-onset time (VOT). Speech sounds ranging from /da/ to /ta/ were presented to each ear at 90 dB SPL. Most patients labeled stimuli presented to the ear ipsilateral to the lesion in a stable and categorical fashion. In contrast, labeling of contralateral stimuli was erratic. Discrimination of VOT pairs was less accurate with the contralateral ear than the ipsilateral one. Three aphasic patients could not label stimuli presented to either ear. Two of these subjects showed normal ipsilateral discrimination but impaired contralateral performance demonstrating a clear dissociation between linguistic and auditory mechanisms. Speech and nonspeech perceptual deficits were uncorrelated.

Deficits in the perception of acoustic transients indicate that cerebral auditory areas play an important and specific role in the processing of certain classes of speech sounds. The contralateral nature of the impairment is consistent with a theory of hemifield organization in the human auditory system.

Supported by grants GT 32 GMO 7484 and RR 00088.

VISUAL CORTEX: EXTRASTRIATE VISUAL AREAS II

280.1 THE ORGANIZATION OF CORTICAL VISUAL AREAS IN A STREPSIRHINE PRIMATE, GALAGO SENEGALENSIS. John Allman and EveLynn McGuinness*, Division of Biology 216-76, California Institute of Technology, Pasadena, California 91125.

The order Primates is divided into two suborders: haplorhines (tarsiers, monkeys, apes, and man) and strepsirhines (galagos, lorises, and lemurs). In order to trace the evolutionary history of the cortical visual areas, we have sought to determine which areas are present in both suborders. We have mapped the representations of the visual field with microelectrode recordings in the visual cortex of a strepsirhine primate, Galago senegalensis. Our results indicate that the primary visual area (V-I), the second area (V-II), and the middle temporal (MT), the dorsolateral (DL), the medial (M), the ventral posterior (VP), the ventral anterior (VA) visual areas are all present in Galago. Previous data indicate that these areas are present in New World monkeys. There is also substantial evidence from other laboratories that most, if not all, of these areas also are present in Old World monkeys. Galago also possesses extensive, visually responsive inferotemporal and posterior parietal cortical regions, but, as in monkeys, receptive fields of neurons in these regions do not appear to be organized in obvious visuotopic maps.

Recently Horton and Hubel (Nature, V. 292, p. 762, 1981) have reported that the system of cytochrome oxidase stained patches in layers II and III of striate cortex are present in Galago as well as New and Old World monkeys but are not present in nonprimates such as cats and tree shrews. The results suggest that there exists a distinctive primate pattern in the functional organization of striate cortex.

Taken together these similiarities in the organization of striate and extrastriate visual cortex in strepsirhine and haplorhine primates suggest that these features were present in the common ancestor of these living forms, which would have been a primitive primate living during the Eocene period 36 to 55 million years ago. These early primates possessed large frontally directed eyes and an expanded posterior neocortex; they were small, probably nocturnal, visually predatory, insectivorous creatures that lived in the fine terminal branches of trees (Cartmill, Science, V. 184, p. 436, 1974; Simons, Primate Evolution, 1972; Radinsky, Am. J. Phys. Anthrop., V. 27, p. 385, 1967). The comparative neurobiological data suggest that many of the distinctive features of the primate visual system developed in these early primates. (Supported by NIH grant EY03851, the Pew Memorial Trust and the L.S.B. Leakey Foundation.)

280.2 SUPPRESSION FROM IPSILATERAL VISUAL FIELD IN AREA V4 OF THE MACAQUE. J. Moran*, R. Desimone, S.J. Schein#, and M. Mishkin (SPON: C. Gross). Laboratory of Neuropsychology, NIMH and #Section on Visual Processing, National Eye Institute, Bethesda, MD. 20205.

Like other prestriate areas, V4 contains a representation of the contralateral visual field. Within at least the central 5°, V4 excitatory receptive fields (RFs) rarely extend more than 1° across the vertical meridian (VM) into the ipsilateral visual field. Yet, V4 receives a heavy commissural projection not strictly limited to the representation of the VM (Van Essen and Zeki, J. Physiol., 1978). We have previously found that V4 RFs have silent suppressive surrounds: Stimuli placed outside the RF are without effect themselves yet are able to suppress the response to an RF stimulus. We report that the suppressive surrounds of V4's RFs extend far into the ipsilateral visual field.

We studied neurons within the representation of the central 5° of V4 in 3 anesthesized (N_2O), paralysed macaques using a semi-chronic preparation. We mapped the RF of each cell by hand and determined the optimal RF stimulus. All RF centers were in the contralateral field, and the mean overlap of the VM was only 0.6°. To test for a suppressive surround, we measured the response to the paired presentation of the RF stimulus and a large annulus (O.D. 21°, I.D. slightly larger than RF) and compared it to the response to the RF stimulus alone. The response of 74% of the cells was significantly suppressed by the annulus. The mean suppression across all cells was 75%. The surround stimulus alone elicited no response. These results were corroborated in one alert monkey, trained to fixate.

To determine if the suppressive surrounds extended into the ipsilateral field, we measured the response to an RF stimulus alone and compared it to the response to an RF stimulus paired with a bar (15° X 20° wide) confined to the ipsilateral visual field. The bar's medial edge was located at 0, 0.5, 1, 2, 4, 8, and 16 degrees into the ipsilateral field. Across all cells suppression was significant out to at least 16°, ranging from 58% at 0° to 18% at 16°.

To determine the contribution of the corpus callosum to the ipsilateral suppression, we sectioned the posterior 1/2 of the corpus callosum in one of the monkeys. Whereas the suppression by an annulus remained equal to that in normal animals, the suppressive effect of the bar confined to the ipsilateral field was significantly reduced. Across all cells suppression was significant only out to 4°; even at 4° the suppression was only half that seen in normal animals.

We conclude that the suppressive surrounds of V4 RFs extend far into the ipsilateral visual field and that the corpus callosum mediates at least part of the suppression.

280.3 DIRECTIONAL PROPERTIES OF PARIETAL VISUAL NEURONS IN MONKEY
M. A. Steinmetz*, C. J. Duffy, B. C. Motter*, and V.B.Mountcastle
The Bard Laboratories of Neurophysiology, Dept. Neuroscience,
The Johns Hopkins Un. Sch. of Med., Baltimore, Md., 21205

The majority of visual neurons in the inferior parietal lobule (area PG) have large, often bilateral receptive fields and respond more vigorously to moving than to flashed stationary stimuli. 90% of the movement sensitive cells respond differentially to stimuli moved in opposite directions through the same zone of their RF's. The "best" directions for 40% of these cells are opposed in the opposite halves of the visual field, pointing either inward or outward with respect to the point of fixation.

The aim of the experiments described here was to study the mechanisms determining the organization of directionalities. For these experiments Macaque monkeys viewing binocularly the central $100^0 x100^0$ of a tangent screen were trained to achieve and maintain fixation of a 0.3^0 target light, and to detect its dimming in order to receive liquid reward. Luminous test stimuli ($10^0 x10^0$) were back projected onto the screen and moved 10^0, 20^0, 40^0 or 100^0, during the fixation period, along the horizontal, vertical, or diagonal axes, at velocities of 30^0/sec to 120^0.sec.

The results indicate that for some parietal visual neurons, the directional-specific response is due to local directional properties within the RF. For other cells, the directionality is the result of the history of the stimulus movement, including movements outside the RF for that cell. For many neurons,both influence the radial organization of the directional responses.

Only rarely are these directional responses evoked by movements along a single axis. More commonly, responses are evoked by stimuli traversing several or even all 4 meridians (8 directions) tested. For many cells, the intensity of response varied with the radial direction of stimulus movement. We have used a sinusoidal regression analysis to estimate a radial directional preference for these cells. Our analyses indicate that all directions are represented in the population of neurons from each hemisphere, with some weighting toward preferred directions into the contralateral field.

These directional properties appear to be ideally suited for a system dealing with spatial relations, and the constancy of the image of the world during movements of head and eyes. In addition these neurons may provide signals for the perception of optic flow patterns associated with translational movements of the body through space, and for the attraction of gaze toward moving objects entering the periphery of the visual field during attention to foveal tasks. This work is supported by Grant #USPHS 5R01EY03167.

280.4 CONNECTIONS OF VISUAL CORTEX ROSTRAL TO THE MIDDLE TEMPORAL AREA (MT) IN OWL MONKEYS: EVIDENCE FOR A VISUAL REGION IN SUPERIOR TEMPORAL CORTEX. R.E. Weller and J.H. Kaas, Depts. of Psychology and Anatomy, Vanderbilt Univeristy, Nashville, TN 37240.

The Middle Temporal Area (MT) of visual cortex has been identified in all primates investigated, and its projections have been described in prosimian galagos, New World marmoset monkeys, and most recently, Old World macaque monkeys. In macaque monkeys, MT projects, in addition to other areas, to bordering cortex adjacent to the representation of the peripheral visual field of MT. We found a similar projection from MT in owl monkeys. Single, restricted injections of ^{3}H-proline in MT of 6 owl monkeys demonstrated terminations in a region of cortex rostral to MT, in the caudal superior temporal gyrus and extending into the caudal end of the temporal sulcus. The projections from MT to this Superior Temporal Region (ST) were bilateral, strongest in granular and supragranular layers of cortex, patchy, and sometimes discontinuous dorsally and ventrally. We investigated the connections of this MT-receptive ST region by placing single, small injections of ^{3}H-proline into dorsal ST in 3 additional owl monkeys, and into ventral ST in 2 other owl monkeys. These injections were judged to be in ST and not in adjacent MT, the Dorsolateral Area (DL), or Inferior Temporal Cortex (IT), from architectonics and the known connections of MT, DL, and IT. While the ST region may project to some areas of visual cortex which also receive input from MT, DL, or IT, ST also projects uniquely to other regions of cortex: 1) a large region of parietal cortex in the caudal Sylvian fissure, extending slightly onto the surface of cortex below the fissure; 2) IT cortex of the ventro-medial surface; 3) cortex in other parts of the temporal sulcus; and 4) all parts of MT. The laminar pattern of projections from ST is different for different target areas, e.g., terminations in the rostral temporal sulcus are concentrated in layer IV, while terminations in MT and the Sylvian fissure are concentrated in layers above and below IV. Injections into dorsal ST result in discontinuous projections to ventral ST, and vice versa, and these interconnections suggest that separate dorsal and ventral subdivisions may exist in ST. However, due to the patchy nature of terminations in the ST area in general, and the many similar connections of dorsal and ventral parts of ST, this suggestion must remain tentative.

Supported by NIH Grant EY02686.

280.5 RESPONSE PROPERTIES AND DISTRIBUTION OF NEURONS WHICH RESPOND TO FACES IN THE MONKEY. C.M.Leonard, E.T.Rolls, G.C.Baylis*, F.A.W.Wilson*, G.V.Williams*, C.Griffiths* and E.Murzi*. Dept. Exptl. Psychol., Oxford Univ., Oxford, England.

Neurons which respond selectively to faces have been found in the cortex in the fundus of the superior temporal sulcus (STS) of the macaque monkey (Perrett, Rolls and Caan, 1982). We are now investigating whether neurons which respond to faces are also found in areas connected to the cortex in the STS, and if so how their responses may differ. In the ventrolateral part of the inferior temporal cortex (area TE) (which projects into the STS), only a small proportion (1% of our current sample) of neurons responded selectively to faces, compared with 20% in the fundus of the STS. The proportion of neurons which responded to faces increased (to 5%) as the recording sites moved into the ventral bank of the STS. In the STS, neurons which responded to faces were found to be distributed over a long anterior-posterior extent which included the anterior and middle thirds of the sulcus (approximately 4 to 16 mm anterior to ear bar zero in the 3-6 kg rhesus and cynomolgous monkeys). Some neurons which responded to faces were also found in the amygdala, which receives projections from temporal neocortical areas. A number of these neurons were found in and around the basal accessory nucleus of the amygdala. These neurons were more likely than those in the STS to differentiate between individuals. In some cases their responses occurred preferentially to categories of faces, such as those of infant monkeys. In the ventral striatum, which receives connections from the amygdala and the temporal lobe neocortex, neurons were found which could respond to faces, but often the emotional significance of the face was important, and some of these neurons also responded to other emotion-provoking visual stimuli.

An analysis of the response properties of face-selective neurons in the temporal neocortex is providing evidence that they respond to different parts of the spatial frequency spectrum present in faces, that responses can occur to a negative image of a face, that in some cases they can respond partly to simple representations of facial features such as two circles to represent the eyes, and that the distance of the face can be important.

These results are consistent with the hypothesis that the brain of the monkey contains a neuronal system in which processing first becomes specialized for parts of faces, then for particular faces or for some types of face, and then becomes appropriate for emotional responses made to such faces.

Perrett DI, Rolls ET, Caan W (1982) Exp Brain Res 47: 329-342.

Supported by The Medical Research Council, Janssen Pharmaceutica, and a NIH senior fellowship to CML.

280.6 OCCIPITAL AND INFEROTEMPORAL RESPONSES TO PHOTIC STIMULI IN THE MONKEY PERFORMING A COGNITIVE TASK. J.W. Ashford* and J.M. Fuster. Dept. Psychiatry and Brain Research Institute, School of Medicine, University of California, Los Angeles, CA 90024.

The purpose of this study was the analysis of neuroelectric responses of striate and inferotemporal cortices to behaviorally relevant visual stimuli. Two such stimuli were presented to the animal at the beginning of each trial in a delayed matching-to-sample task. The first was a brief (10 μsec) diffuse white flash to alert the animal. The second stimulus (the sample), which followed 2 sec later, was a circular colored light (about 8^0, red or green) and lasted for 1.5 sec. The animal had to remember it for a color match at the end of a subsequent delay (10 sec). The two monkeys in the study consistently performed the task better than 90% correctly. Stimulus-related activity of small groups of neurons and local field potentials were recorded with microelectrodes (100-500 kΩ impedance).

In striate cortex, the alerting flash elicited cell-activity changes within 16 msec and field-potential changes within 20 msec. Excitatory cell changes predominated for at least 100 msec after flash. Cells differed with regard to latency and duration of firing change, but the sum of unit discharge across the cortex showed a pattern suggestive of the integral of the evoked potential. Inferotemporal cells were predominantly inhibited by the flash, yet the inferotemporal potential was similar to that of the occipital area, though delayed by 20-40 msec.

Changes evoked by the sample stimulus had longer latency than those evoked by the flash. In striate cortex, cell responses began about 50 msec after sample-onset and the field potential about 10 msec later. Most responding cells showed an excitatory on-reaction followed by sustained firing elevation while the light remained on. Many units in inferotemporal cortex also showed clear responses to the sample, but the onset of those responses usually had a longer latency than that of striate cells. Here also the field potential response trailed the unit response.

The results show in both cortical areas a loose general relationship between unit activity and field potentials. Latency differences suggest that inferotemporal cortex is engaged after striate cortex in the processing of a given stimulus. However, the time course of the observed changes indicates that processing takes place to a large extent jointly and simultaneously in the two structures, possibly involving the reciprocal connections that unite them. The results also suggest that the inferotemporal cortex is more attuned to stimuli with relevant detail than to those without it.

280.7 ACTIVITY OF HUMAN MEDIAL TEMPORAL LOBE NEURONS DURING VISUAL FIXATION. C.L. Wilson, T.L. Babb and P.H. Crandall. Brain Res. Inst., Dept. of Neurology and Dept. of Surg/Neurol., UCLA Sch. of Med., Los Angeles, CA 90024.

Seltzer and Van Hoesen (Br. Res. 178:157-161, 1978), have described a direct projection from the inferior parietal lobule to the presubicular area in monkey. We report here the results of recordings from 12 units in the posterior parahippocampal gyrus, immediately adjacent to presubiculum, from a complex-partial epilepsy patient during depth electrode studies aimed at localizing suspected areas of focal epileptic discharge.

Eight of these units displayed some of the response characteristics of neurons studied by other investigators of the monkey posterior parietal area. All were at least weakly visually responsive to various photic stimuli, but no receptive fields could be identified in spite of prolonged mapping attempts. The response of these cells was most prominent during visual fixation of partial areas within the patient's visual field. In 6 of these units, these areas were organized on the basis of response gradients in which moving gaze from a central fixation point to the upper visual field increased or decreased rate of firing, while moving the gaze to the lower portion of the central fixation visual field produced the opposite effect. Four units increased firing during fixation in the upper field and 2 during fixation in the lower field. Cellular discharge rate was independent of changes in luminance level in these portions of the visual field. Visual responsiveness was binocular in all these units.

A pronounced acceleration of firing rate occurred during total darkness or eye closure in 6 units, and firing rate was unaffected by eye movement to areas of the field which had suppressed firing under either well lighted or even dimly lighted scotopic conditions. Fixation of dim red LEDs in otherwise total darkness effectively modulated cellular discharge as it had during normal lighting. Saccades away from an excitatory fixation area sometimes accelerated firing until fixation in an area which suppressed discharge. Saccades into an excitatory area of the visual field were not accompanied by firing and the latency of firing after fixation was sometimes one sec or more.

Neurons displaying some of the characteristics of these units have been reported by Mountcastle and his co-workers and by others including Sakata et al. (J. Neurophysiol., 43:1654-72, 1980) to which they bear the most resemblance. Recordings by both of these groups included the posterior parietal lobule or 7a in the monkey. Reciprocal connections between parietal and limbic areas have been cited as a possible source of some of the motivational-attentional deficits resulting from damage in this area.

Supported by NS 02808 NIH.

ACETYLCHOLINE: BIOSYNTHESIS AND REGULATION

281.1 EVIDENCE FOR TWO FUNCTIONAL DOMAINS OF CHOLINE ACETYLTRANSFERASE IN THE NEMATODE C. ELEGANS. J.B. Rand and R.L. Russell*, Dept. of Biological Sciences, Univ. of Pittsburgh, Pittsburgh, PA 15260.

The gene cha-1 on linkage group IV is the structural gene for choline acetyltransferase (ChAT). Four severe mutant alleles of this gene are known, which all lead to the same range of phenotypes: low ChAT activity (<2% of wild-type), characteristic uncoordinated jerky behavior, small size; slow growth, and resistance to cholinesterase inhibitors. Very tightly linked to cha-1 is the gene unc-17, mutants in which were originally identified by their uncoordinated behavior. Mutations at unc-17 lead to behavioral and developmental phenotypes very similar to those of cha-1 strains (including the jerky movement and resistance to cholinesterase inhibitors), except that unc-17 strains have apparently normal ChAT levels.

Recent experiments have indicated that cha-1 and unc-17 are both part of a single complex gene. Mutations at this locus fall into one of three categories: "pure" cha-1 alleles and "pure" unc-17 alleles form two disjoint complementation groups, but there are three "overlap" alleles which behave as members of both complementation groups (as measured by uncoordinated behavior). It is especially noteworthy that two of these "overlap" alleles are unc-17-like in that they lead to apparently normal ChAT levels, yet they fail to complement the behavioral phenotype of the enzyme-deficient cha-1 strains.

We interpret these and other data to support the notion that the ChAT protein has two functionally independent and structurally discrete domains: one contains the ChAT catalytic site, and the other domain is probably involved in the specific subcellular localization of the ChAT molecule. The cha-1 mutations are known to disrupt the catalytic site of the enzyme (based on altered Km) and we postulate that the unc-17 mutations affect the localization function. Such a model suggests that uncoordinated behavior can result from either improper localization of ChAT or inadequate catalysis; the fact that mutations specifically affecting one domain complement those specially affecting the other suggests that the enzyme functions normally in vivo as a dimer. Presumably, the overlap mutants with ChAT activity would be defective in the localization function and also unable to dimerize. We have found that some unc-17 strains have altered ratios of the known multiple forms of ChAT, and using a combination of biochemistry and genetics, we hope to elucidate the molecular basis of this non-catalytic yet behaviorally essential function of the ChAT molecule.

281.2 MONOCLONAL ANTIBODIES TO CHICK CHOLINE ACETYLTRANSFERASE. C.D. Johnson* and M.L. Epstein. Departments of Zoology and Anatomy, University of Wisconsin, Madison, WI 53706.

Monoclonal antibodies were made against choline acetyl transferase (EC 2.3.1.6, ChAT) isolated from the optic tectums of chickens. ChAT was purified approximately 1000-fold by a combination of precipitation with polyethylene glycol and fractionation on a DEAE column. Balb/c mice were immunized with the ChAT preparation. The spleen was removed from an immunized mouse and fused with mouse myeloma NS1 cells. The resulting hybridomas were screened as follows: Immulon plates were coated with antisera against mouse immunoglobulins (IgA, IgG, IgM). The plates were incubated with culture supernants, washed, incubated with a small amount of ChAT and washed. The presence of bound ChAT was deteoted according to the method of Rand and Johnson (Anal. Biochem. 116: 361). Six of the hybridomas were cloned by the method of limiting dilution, and all were found to be IgG. These hybridomas were subsequently injected into mice to produce ascites fluid. The immunoglobulins from the ascites were precipitated with 50% $(NH_4)_2 SO_4$, dialyzed, and further purified by affinity chromatography using immobilized anti-mouse IgG. The purified IgG from one clone (1 AG/FI) was subsequently reacted with Affigel 10 to generate an anti-ChAT affinity column. The partially-purified preparation of ChAT was passed over the anti-ChAT-affinity column. Elution of the column with pH 2.2 buffer yielded an active enzyme, which gave one band with an apparent molecular weight of 68 k daltons on an SDS-PAGE gel. These antibodies should be useful for studies of ChAT in the developing chick. Supported by NIH AM 32978.

281.3 CHARACTERIZATION OF CHOLINE ACETYLTRANSFERASE BY IMMUNOBLOT AUTORADIOGRAPHY. J.H. Peng, E.G. McGeer and P.L. McGeer (SPON: D. Paty). Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver, B.C. Canada V6T 1W5

The immunoblot autoradiographic technique was employed to evaluate the specificity and cross-ractivity of anti-human brain choline acetyltransferase (ChAT, EC 2.3.1.6) monoclonal and polyclonal antibodies. The procedure involves SDS-polyacrylamide gel electrophoresis of crude and pure ChAT preparations, transfer of proteins from SDS-gel on to nitrocellulose paper, incubation with specific monoclonal and polyclonal antibodies, followed by incubation with ^{125}I-labelled protein A or ^{125}I-labelled goat anti-rabbit or anti-mouse Ig, and finally visualization by autoradiography. Rabbit or mouse anti-human brain ChAT was shown to cross-react with ChAT from brains of human, monkey, bovine, guinea pig, rabbit, rat and chicken and from human placenta. The molecular weight of ChAT from these mammalian and chicken brains and human placenta were very similar, around 66,000 daltons. Mouse and rat brain preparations also contain one or two minor protein bands which cross-reacted with the antibodies indicating other forms of ChAT in these two species. This study further substantiates the monospecificity of our rabbit and mouse anti-human brain ChAT antibodies.
(Supported by the Medical Research Council of Canada.)

281.4 MECAMYLAMINE POTENTIATES THE LITHIUM-INDUCED INCREASE IN RAT BRAIN myo-INOSITOL-1-PHOSPHATE, M.P. Honchar and W.R. Sherman*, Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110, U.S.A.

Administration of lithium (Li) to rats produces an increase in levels of D-myo-inositol-1-phosphate (MIP), which is a product of phosphoinositide metabolism (Sherman et al., J. Neurochem 36, 1947, 1981). Muscarinic cholinergic receptors are involved since atropine, a muscarinic antagonist, blocks the MIP response (Allison et al., BBRC 71, 664, 1976). We have extended these studies to investigate the interaction of Li with nicotinic receptors in rat cerebral cortex. Rats received 3 meq/kg LiCl and were sacrificed 26 hrs later. Other drugs were administered at the following doses and times before sacrifice: mecamylamine (Mec) 50 mg/kg, 2 hrs; pilocarpine (Pilo) 30 mg/kg, 1 hr; atropine 150 mg/kg, 2.5 hrs.

Mecamylamine alone produced only a slight effect on MIP levels when administered alone, however, when administered with Li a ten fold increase occurred (3-fold above Li alone). Pilo administered with Li and Mec produced no further enhancement while atropine prevented the increase in MIP.

The results show that Mec, a centrally-active ganglionic nicotinic inhibitor, has an effect on phosphoinositide metabolism that is similar to a muscarinic agonist, Pilo. This would be consistent with the involvement of a Renshaw cell-like inhibitory process in the Li/MIP effect. Supported by NS-05159.

TABLE: MIP in midline cerebral cortex (mmol/kg dry wt ± SEM, N=5)

Control	0.24 ± 0.02
Mec	0.32 ± 0.04
Li	0.75 ± 0.13
Mec + Li	2.37 ± 0.31
Mec + Atropine + Li	0.36 ± 0.02
Atropine + Li	0.23 ± 0.02
Pilo	0.40 ± 0.02
Pilo + Mec	0.33 ± 0.04
Pilo + Mec + Li	6.55 ± 0.29
Pilo + Li	7.96 ± 0.84

281.5 CHOLINE ACETYLTRANSFERASE ACTIVITY IN THE RAT BRAIN FOLLOWING ADRENALECTOMY. N.S. Nadi* and D.C. Jimerson, Section on Psychogenetics and Laboratory of Clinical Science, NIMH Bethesda, Maryland 20205

Using ^{3}[H] quinuclidinyl benzilate (QNB), we previously demonstrated a significant decrease in the muscarinic cholinergic binding sites in the main olfactory bulb (MOB) of male Sprague-Dawley rats 7-9 days following adrenalectomy (500 ± 124 vs 1140 ± 130 fmol/mg protein). Other areas of the brain studied, such as the amygdala, hypothalamus, hippocampus, cortex and cerebellum showed no significant differences in the binding of ^{3}[H]QNB. There were no changes in affinity or receptor number for GABA or beta-adrenergic sites in the MOB or other brain areas studied. In the present study we measured the effects of adrenalectomy on the activity of choline acetyltransferase (CAT) in the MOB and other brain areas indicated above. Rats were sacrificed 7-9 days post-adrenalectomy, brain regions were dissected and frozen. CAT was evaluated according to the method of Wilson et al. (J. Biol. Chem., 247, 3159, 1972). The results indicate that there is a significant increase in CAT activity in the MOB following adrenalectomy (7.4 ± 0.8 nmol/mg protein/15 min., as compared to controls 5.1 ± 0.4 nmol/mg protein/15 min. ($p < 0.05$)). In the hypothalamus, a significant decrease in the activity of CAT was seen (9.1 ± 2.9 nmol/mg protein/15 min. following adrenalectomy, 27.8 ± 5.04 nmols/mg protein/15 min. in controls, $p < 0.01$). The other brain areas analyzed for CAT showed no significant differences between controls and adrenalectomized animals. The results observed in the MOB suggest that the reduction of the number of QNB receptors seen in earlier experiments is associated with increased activity of presynaptic cholinergic nerve terminals in the MOB. Further experiments are necessary to determine whether the receptor alterations are primary, or secondary to increased activity of cholinergic afferents to the MOB. In the hypothalamus, in contrast to the MOB, there appears to be a dissociation between altered CAT activity and stable muscarinic receptor number post-adrenalectomy. These results suggest that reciprocal interactions between pre- and post-synaptic cholinergic function following adrenalectomy may reflect unique properties of the MOB.

281.6 DOSE DEPENDENT BRAIN NEURONAL RNA AND BEHAVIORAL RESPONSES IN SOMAN TOXICATED RABBITS, J. Doebler*, T. Wall*, and A. Anthony* (SPON: J.J. Valdes). Penn State Univ., Univ. Park, PA 16802.

Acute (20 min-1h) and subacute (6-8h) acetylcholinesterase (AChE) and RNA responses were monitored in soman toxicated rabbits (20,30 or 40 µg/kg). Scanning-integrating microdensitometry was used to quantify AChE activity and RNA contents of individual cerebrocortical (Layers III and V) and caudate neurons. In addition, plasma and RBC ChE levels were measured using an automated colorimetric procedure, and particular attention was placed on overt symptoms of toxication.

AChE activity in brain and blood was markedly depressed in a dose dependent manner; there was little enzyme restitution in animals sacrificed 6-8h following toxication. Neuronal RNA was depressed or unchanged in the acute phase using 20 or 30 µg/kg, and invariably reduced using 40 µg/kg soman. Additional, more severe RNA depletion was evidenced 6-8h following 30 µg/kg soman, but not with 20 µg/kg. Typical symptomatic responses were as follows: with 20 µg/kg, marked behavioral arousal was evidenced in the acute phase; with 30 µg/kg, forced circling movements and/or prostration were evidenced at 1h, whereas deep stupor, hyporeflexia, cardiovascular-respiratory impairment and often death were evidenced at 6-8h; 40 µg/kg soman produced forced circling movements, clonic convulsions and death at ca. 20 min.

These findings indicate that soman, at lethal or near-lethal doses, produces metabolic correlates of impaired rather than accentuated activation of brain cholinergic compartments. RNA depletion is more prominent with lethal doses and is more severe in the depressant phase of toxication observed after the disappearance of manifestations of cholinergic hyperexcitation. It is postulated that the observed behavioral responses stem from biphasic actions on cholinergic reticulocortical pathways known to play an important role in attention, awareness and consciousness; i.e., at low doses such pathways are activated producing arousal, whereas at high doses function is disrupted resulting in depression. Impaired neuronal activation may stem from blocking or curare-like actions of acetylcholine, which could also precipitate central respiratory distress both in acute and subacute stages of toxication. (Supported in part by USAMRDC Grant DAMD 17-81-C1202).

281.7 PURIFICATION OF BOVINE CHOLINE ACETYLTRANSFERASE. S. M. Hurd* and W. O. McClure. Section of Neurobiology, Univ. So. Calif., Los Angeles, CA 90089-0371.

Choline acetyltransferase (CAT) from bovine caudate nuclei has been purified to give two distinct molecular forms by a modification of the technique of Ryan and McClure (Biochem. (1979) 18:5357). The basis of the technique is an affinity gel which uses coenzyme A as the ligand. This gel is used with two different elution schemes. First, the enzyme is eluted with a linear 0-1.5 M NaCl gradient. The pool is desalted and applied to a second CoA-Sepharose column, which is eluted with a linear gradient of 0-100 μ M coenzyme A. The active fractions are immediately collected on an Amicon Blue A column. This column is eluted with a step gradient of 1.5 M NaCl pH 7.2.

At this level of purity the enzyme shows two bands on SDS-polyacrylamide gels corresponding to molecular weights of 65 and 68 kdaltons. These same two bands are seen when the enzyme is purified using immunoaffinity techniques (Hurd et al. (1983) Transact. Am. Soc. Neurochem.). On the basis of this immunological cross-reactivity, as well as the specificity the two forms show in binding to the CoA ligand, it would seem that they are two different molecular forms of CAT. These two forms cannot be identified with the two isozymes, Bov I and Bov II, which have been previously reported in the literature.

Preliminary analyses indicate that the kinetic parameters obtained with this enzyme preparation are essentially identical to those found in the immunoaffinity purified enzyme and to those found by earlier authors.

This work has been funded, in part, by grants from the NIH and from Nelson Research.

281.8 STRUCTURAL DISPARITY BETWEEN CATALYTIC SUBUNITS OF 6S AND 17S ACETYLCHOLINESTERASE FROM TORPEDO CALIFORNICA, H.W. Chang*, A. Kim* and E. Bock* (SPON: S. DiMauro). Dept. of Neurology, Columbia University, College of Physicians & Surgeons, New York, NY 10032.

Acetylcholinesterase (AChE) in the electric organ of Torpedo californica exists in two principal molecular forms, 17S and 6S, which differ in their mode of membrane associations. Comparison of our amino acid analysis data from the isolated catalytic subunits of the detergent soluble 6S and the high salt soluble 17S AChE suggests that the catalytic subunits of the two forms of enzyme are not identical. We have therefore analyzed the degree of divergency between the catalytic subunits with the aid of peptide mapping. When the isolated catalytic subunits of 6S and 17S AChE, 66K and 70K dalton respectively, were partially digested with various proteolytic enzymes and subjected to polyacrylamide gel electrophoresis in SDS, many protein bands common to both subunits were observed. However, many of the prominent bands are in fact unique to one or the other of the subunits. Upon digestion with elastase, the most striking difference is the presence of a doublet of 40K and 41K dalton in the 66K subunit which is absent in the 70K subunit. Instead, the 70K subunit contains a single band of slightly higher molecular weight. Furthermore, with increasing elastase concentration a broad polypeptide band of 29K and 31K dalton is generated from the 66K and 70K subunit respectively. Peptide maps of 66K and 70K catalytic subunits generated by Streptomyces griseus protease also gave distinct peptide bands, 28K and 32K dalton respectively. When in a similar experiment the catalytic subunits were digested with papain, again distinctive peptide fragments unique to each subunit were observed. At higher papain concentration many small fragments common to both subunits were generated. However, a broad 12K dalton band was generated only from the 70K subunit. In contrast to these protein stained peptide maps, autoradiographs of peptide maps which reveal only those peptide fragments bearing the [^{3}H]DFP labeled active sites, showed a high degree of homology between the two subunits. None of the distinct peptide fragments generated by the three proteases unique to each subunit appeared on the autoradiograph. These results suggest that the active sites of these catalytic subunits are highly conserved - perhaps derived from a common ancestral gene - and that the differences lie in peripheral noncatalytic regions of the subunits. In summary, the 17S enzyme differs from the 6S enzyme not only in possessing two extra non-catalytic subunits, but also in the structure of the catalytic subunit itself. The 6S enzyme being composed only of catalytic subunits, must contain within these subunits the amino acid sequences which dictate its localization as a hydrophobic integral membrane protein.

Supported by NSF PCM 80-22832, NS-13744, and the Muscular Dystrophy Association of America.

281.9 DISRUPTION OF CHOLINE METABOLISM: A PROPOSED MECHANISM FOR THE CYTOTOXIC EFFECT OF AF64A, A CHOLINERGIC NEURON-SPECIFIC NEUROTOXIN. K. Sandberg, R.L. Schnaar, I. Hanin, A. Fisher* and J.T. Coyle. Depts. of Neuroscience, Psychiatry, Pharmacology, Div. Child Psychiatry, Johns Hopkins Univ Sch Med, Baltimore, MD 21205 and the Depts of Psychiatry and Pharmacology, University of Pittsburg School of Medicine, Pittsburg, PA 15260.

Ethylcholine mustard aziridinium ion (AF64A) is a reactive choline analog. In cell culture studies, AF64A causes selective cytotoxic effects on the cholinergic neuroblastoma x glioma hybrid cell line NG-108-15 with marked inhibition of high affinity choline transport (HAChT) and choline acetyltransferase (CAT) activity occurring more rapidly, than cell death (Sandberg, K. et al., Soc. Neurosci. Abstr. 8:516, 1982). In the present study, the mechanism of AF64A cytotoxicity was further investigated to determine if the drug acts as an irreversible inhibitor of enzymes involved in choline metabolism.

The effects of AF64A on several partially purified enzymes were examined. When the enzyme was preincubated for 10 min with 50 uM AF64A, the drug inhibited CAT activity by 85 + 4%. The same drug treatment caused inhibition of choline kinase activity by 90 + 4% and acetylcholinesterase activity by 60 + 4%. In contrast, these conditions resulted in less than 12% inhibition of enzymes which do not use choline as a substrate including alcohol dehydrogenase, carboxypeptidase A, chymotrypsinogen, and lactate dehydrogenase. The inhibition of partially purified CAT could not be reversed by dialysis for 12 hours followed by chromatography of the treated enzyme on a Sephadex G-25 column. However, co-incubation of CAT and AF64A (50 uM) with choline (500 uM) protected the enzyme against the inhibitory effects of the drug. The above findings raised the possibility that inhibition of choline metabolism might contribute to the cytotoxic action of AF64A. Therefore, phospholipids were extracted from cell pellets with ethanol/ether, separated by isocratic HPLC on a silica column, and quantitated by measuring absorbence at 214 nm. The cellular concentration of phosphatidylcholine (PC) decreased (35+4%), phosphatidylethanolamine (PE) (30+4%) and phosphatidylserine (PS) (22+3%) after 24 hours continual exposure of the cells to 50 uM AF64A. Choline kinase, the first enzyme involved in a major pathway for PC synthesis was inhibited by 45+3% after treatment of the intact cells for 12 hours with 50 uM AF64A. Addition of choline (500 uM) to the medium both enhanced cell survival and reduced the effect of AF64A on phospholipid concentrations. These results indicate that AF64A enters the cell via HAChT inhibits enzymes involved in choline metabolism and causes a decrease in the cellular concentration of PC which may cause disruption of the plasma membrane and cell death. Supported by USPHS Grant NS-18414 and MOD Grant 5-302.

281.10 CHANGES IN MOLECULAR FORMS OF BRAIN ACETYLCHOLINESTERASE AFTER DIISOPROPYLFLUOROPHOSPHATE TREATMENT. S. C. SUNG AND BLAIR A. RUFF*. Division of Neurological Sciences, University of British Columbia, Vancouver, B. C., Canada, V6T 1W5.

Diisopropylfluorophosphate (DFP) inhibits acetylcholinesterase (AChE) irreversibly and de novo synthesis of the enzyme must take place for AChE activity to reappear. Regeneration of molecular forms of AChE in rat brain subcellular fractions following DFP treatment has been investigated. 1-2 hr after I.M. injection of 1.5 mg of DFP/kg of body weight, the AChE activities in cerebellum, cortex, midbrain and striatum of rat brain decreased to 18-29% of the controls. The most severe drop in AChE activity was observed 1-2 hr after DFP treatment. Only partial recovery was observed on day 7 after DFP, and almost complete recovery was seen on day 21 after DFP. The AChE activities found in P_1 (nuclear), P_2 (synaptosomal) and P_3 (Microsomal) fractions were composed mostly of 10 S form with very little 4 S form. Therefor, the major activity found in these fractions after DFP treatment was 10 S form, being less than 25% of the controls. Among these fractions the greatest decrease was observed at 1 hr after DFP in the P_3 fraction, however, on the contrary, somewhat increased activity of AChE was found in the P_3 fraction on day 21 after DFP. The pattern of changes in AChE activity in the S_3 (100,000 x g supernatant) fraction from the striatum differed at various stages of recovery. At 1 hr after DFP, drastical decrease of both 4 S and 10 S forms was observed. On day 7 after DFP, the decrease in 4 S form seemed to be more severe than that in 10 S form. By day 21 after DFP treatment, nearly normal pattern in distribution of 4 S and 10 S forms of AChE in the S_3 fraction was resumed.

Supported by the Medical Research Council of Canada.

281.11 IONIC REGULATION OF [^{3}H]PIRENZEPINE AND [^{3}H](-)QUINUCLIDINYL BENZILATE BINDING IN RAT CEREBRAL CORTICAL AND MYOCARDIAL HOMOGENATES. Mark Watson*, William R. Roeske* and Henry I. Yamamura. Departments of Pharmacology, Biochemistry, Internal Medicine, Psychiatry and the Arizona Research Laboratories, University of Arizona, Health Sciences Center, Tucson, AZ 85724.

The non-classical antagonist [^{3}H]pirenzepine ([^{3}H]PZ) selectively identifies putative M_1 muscarinic receptor binding sites in the rat cerebral cortex (Life Sci., 31:2019, 1982). This high affinity [^{3}H]PZ binding which exhibits a K_d=8.5nM may be best described by two sites with K_ds of 3.0nM and 207nM respectively. We have reported that [^{3}H]PZ binds to rat cerebral cortical homogenates with a K_d of 2-3nM in 10mM Na-K phosphate buffer at 25°C using a rapid filtration assay, and that while ions exert significant effects upon [^{3}H]PZ binding, guanine nucleotides do not (Life Sci., 32:3011, 1983). We have examined the binding of [^{3}H]PZ and [^{3}H](-)quinuclidinyl benzilate ([^{3}H](-)QNB) in 10mM Na-K phosphate buffer in parallel assays with 50mM Na-K phosphate buffer and Krebs phosphate buffer at 25°C in homogenates of the rat cerebral cortex which is largely M_1 and the rat myocardium which is predominately M_2. Cortical [^{3}H]PZ binding decreased with increasing ionic strength of the buffers, producing K_ds of 2.8nM in 10mM, 6.8nM in 50mM and 10.5nM in Krebs buffer. A similar, though less apparent, effect was seen in the heart, and 10mM Na-K phosphate buffer produced the highest B_{max} values in both tissues. [^{3}H](-)QNB bound with a K_d of 15pM and 18pM in 10mM, 31pM and 22pM in 50mM, and 30pM and 27pM in Krebs buffer in the cortex and heart respectively, showing equal affinity for muscarinic sites in both tissues. However, cortical B_{max} values showed increases from 77 fm/mg tissue in 10mM, to 92 fm/mg tissue in 50mM, and to 100 fm/mg tissue in Krebs buffer, whereas this effect was not apparent in the heart. Interestingly, while our kinetic data for [^{3}H]PZ binding at 25°C is in reasonable agreement with values from our equilibrium binding studies, we have obtained data using 0.2 nM [^{3}H]PZ with cortical homogenates in 10mM Na-K phosphate buffer which yields an association rate (k_{+1}) equal to 5.0×10^8 M^{-1} min^{-1} and a dissociation rate (k_{-1}) of 3.6×10^{-2} min^{-1} indicating a kinetic dissociation constant (k_{-1}/k_{+1}) of 72pM. We are presently completing experiments to determine if receptor isomerization occurs. Though ions modulate [^{3}H]PZ and [^{3}H](-)QNB binding in both putative M1 and M2 tissues, they do not appear to interconvert these sites. Pirenzepine seems to discriminate between antagonist states modulated by molecules associated with the receptor or small intrinsic differences in the primary structure of the receptor molecule itself. (Supported by USPHS grants).

281.12 NICOTINIC ACETYLCHOLINE RECEPTOR-LIKE ANTIGENIC DETERMINANTS AND α-BUNGAROTOXIN BINDING SITES IN RAT BRAIN. R. J. Lukas. Division of Neurobiology, Barrow Neurological Institute, 350 West Thomas Road, Phoenix, Arizona 85013.

Two polyclonal antisera (pcas) raised against Electrophorus electroplax nicotinic acetylcholine receptor (nAcChoR), and three monoclonal antibodies (mcab) directed against Torpedo electroplax nAcChoR are characterized using four different types of radioimmunoassay (RIA) and Torpedo nAcChoR as the test antigen. Ability of both anti-nAcChoR pcas and one mcab to inhibit binding of 125-I-labeled α-bungarotoxin (I-Bgt) to nAcChoR is also established. A polyclonal anti-Bgt antisera is also used to provide background information on RIA specificity toward toxin binding sites.

Anti-nAcChoR and anti-Bgt pcas and mcab are tested for cross reactivity toward high affinity I-Bgt binding sites and nAcChoR-like antigenic determinants in rat brain. In an immunoprecipitation assay, anti-Bgt quantitatively precipitates Bgt:toxin binding site complexes, while only a low level of brain Bgt sites are precipitated by one anti-nAcChoR pcas, but not the other pcas, or any of the mcab. Nevertheless, specific binding of I-Bgt to brain membranes that are recognized by anti-nAcChoR pcas is detectable, and both anti-nAcChoR pcas and one mcab inhibit high affinity I-Bgt binding to both brain and Torpedo membranes. When antibody binding to brain membranes is measured via 125-I-labeled protein A binding paradigms, the presence of antigenic determinants is detected, at concentrations in excess of I-Bgt binding sites, for all three mcab and for both anti-nAcChoR pcas. The results suggest that when adequately sensitive assays are used, cross-reactivity of anti-electroplax nAcChoR antibodies with brain Bgt binding sites are evident. Thus, antibodies/antisera prepared against nAcChoR from peripheral tissues may be useful as probes for rat brain Bgt binding sites, and for nAcChoR-like entities that do not bind Bgt with high affinity.

Supported by NIH grant NS-16821, the Epilepsy Foundation of America, Epi-Hab of Arizona Inc., and the Men's and Women's Boards of the Barrow Neurological Foundation.

281.13 EFFECTS OF PARAOXON ON THE POSTMORTEM RELEASE OF CHOLINE FROM RAT BRAIN. C. J. Flynn and L. Wecker. Dept. of Pharmacology, Louisiana State University Medical Center, New Orleans, LA 70112.

Data suggest that that the organophosphate cholinesterase (ChE) inhibitors may alter the disposition and metabolism of choline and choline-containing compounds in brain. The organophosphates increase the steady-state concentration of acetylcholine (ACh) but little is known about the effects of these agents on other choline-containing compounds. Since the magnitude of the increase in the levels of ACh induced by the organophosphate paraoxon has been shown to be related to the endogenous availability of choline, it was of interest to determine the effects of paraoxon on the production of choline postmortem in brain. Male Sprague-Dawley rats (160-250 g) were injected with either saline or paraoxon (0.23 mg/kg,s.c.,a dose that produces 95% inhibition of acetylChE in brain) and sacrificed 60 min after injection by either head-focused microwave irradiation or decapitation. Brains were quickly removed and either immediately chilled, dissected, weighed and homogenized (microwaved rats) or incubated for 5,10,15 or 30 min at 37°C in a moist chamber. Samples were then prepared for the analysis of the concentrations of choline and ACh or phospholipids. The endogenous concentrations of ACh (microwaved rats) in striatum from control and paraoxon-injected rats were 99 and 133 nmoles/g tissue, respectively. ACh levels decreased linearly for 10 minutes at a rate of 7.9 nmoles/g/min in controls and 10 nmoles/g/min in paraoxon rats, reaching a minimum at 10 min for both groups. By 30 min, the levels of ACh in the striatum from saline and paraoxon-injected rats were 15.8 and 24.4 nmoles/g, respectively. The postmortem production of choline was linear in both groups for 15 min. In saline-injected rats, the rate of increase was 43.5 nmoles/g/min with maximal values of 717 nmoles/g at 15 min. In paraoxon-treated rats, the rate of increase of choline was 22.5 nmoles/g/min, with maximal values of 710 nmoles/g by 30 min. No changes were detected in the postmortem levels of choline-containing phospholipids. Data suggest that paraoxon may affect the rate of choline production through inhibition of the enzymes mediating the postmortem production of choline. (Supported by USDHHS grants NIMH 33443 and NIEHS 02278, and contract DAMD17-83-C-3012 from the U.S. Army.)

282.1 ORGANIZATION OF THE NUCLEUS BASALIS PROJECTION TO CEREBRAL CORTEX IN THE RAT: CHOLINERGIC PATHWAYS VIII. C.B. Saper (SPON: J. Trotter). Dept. of Neurology, Washington University School of Medicine, St. Louis, Missouri 63110.

The detailed organization of the nucleus basalis (NB) projection to the cerebral cortex in the rat was studied using a variety of anterograde and retrograde transport methods. Following an extensive series of injections of wheat germ agglutinin-horseradish peroxidase conjugate into different cerebral cortical areas, the distribution of retrogradely labeled NB neurons was seen to be most closely related to the topography of anterogradely labeled cortical efferent fibers entering the diencephalon. Descending cortical fibers could even be followed into apparent anterogradely labeled terminal fields among the dendrites of NB neurons retrogradely labeled by the same injection. NB neurons projecting to cortical areas whose axons enter the diencephalon through the fornix are therefore primarily found in the adjacent medial septum-diagonal band while NB neurons innervating lateral neocortical areas whose axons pass through the globus pallidus on their way to the thalamus are found primarily in the globus pallidus and adjacent substantia innominata. NB neurons projecting to olfactory cortex, whose fibers enter the diencephalon through the inferior thalamic peduncle, are primarily seen in the adjacent magnocellular preoptic nucleus. Considerable topography in relation to descending cortical fibers can also be demonstrated within these NB clusters. Injections of different fluorescent dyes into multiple cortical areas in single animals confirm this distribution, and indicate that NB neurons have terminal fields, for the most part, of less than 2mm in extent.

Injections of tritiated amino acids into NB has allowed the autoradiographic tracing of the course of its fibers innervating cortex. A medial pathway passes through the septum and around the genu of the corpus callosum to innervate medial cortical structures, while a lateral pathway passes ventrally through the striatum and substantia innominata into the external capsule to distribute to lateral cortical structures. With minor variations, the labeled fibers were found in highest density in the deep cellular layers (eg, layer V of neocortex, layer II in olfactory cortex). Little labeling was found in superficial cellular layers, but moderate labeling of the superficial molecular layer was seen in most areas.

NB provides a highly topographically organized projection mainly to layers I and V of the rat neocortex. Its spatial dispersion in the basal forebrain is explained by its close relationship with the organization of descending cortical fibers.

Supported by grants from NINCDS (NS 18669 and NS 0631), the McKnight Foundation and the American Parkinson Disease Assoc.

282.2 IDENTIFICATION OF CHOLINERGIC PROJECTIONS TO CORTEX USING COMBINED RETROGRADE TRACING AND IMMUNOHISTOCHEMICAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE: CHOLINERGIC PATHWAYS IV. D.B. Rye*[a], B.H. Wainer[a], M.-M. Mesulam[b], and C.B. Saper[c]. (SPON: M. O Shea). Depts. of Pathology and Pediatrics, The Univ. of Chicago, Chicago, IL., 60637[a]; Dept. of Neurology, Havard Medical School, Beth Israel Hosp., Boston, MA.,02215[b]; and Dept. of Neurology, Washington Univ. School of Medicine, ST. Louis, MO. 63110[c].

The present report describes preliminary studies on the topography of cortical cholinergic projections by co-visualizing retrograde horseradish peroxidase-wheat germ agglutinin (HRP-WGA) and choline acetyltransferase (ChAT). Sections from rat brains injected with HRP-WGA were perfusion-fixed and developed for retrograde tracer and ChAT-staining either separately or in series. HRP-WGA was developed with DAB intensified by cobalt, and ChAT localization was performed using immunoperoxidase and monoclonal antibody Ab8 (J. Neurosci. 3:1-9,1983). In sections processed for both markers, double labeled cells exhibited a black granular staining (HRP-WGA) and homogenuous brown cytoplasm (ChAT). Review of appropriate control series indicated that the two histochemical procedures did not markedly alter each other. Rostral to caudal injections into the major areas of neocortex revealed a predominantly rostral-caudal pattern of labeling within the substantia innominata-nucleus basalis of Meynert (SI-NBM)(Ch4). In double-stained series the majority of retrogradely labeled cells stained positively for ChAT. Injections into hippocampus and olfactory bulb revealed predominant labeling throughout the medial septal-diagonal band and horizontal limb-magnocellular preoptic complexes, respectively. ChAT-positive cells were observed in double-stained series, however a substantial proportion of retrogradely labeled cells were ChAT-negative. These findings suggest that basal forebrain-allocortical projections contain a significant non-cholinergic component. This work was supported by grants from the Whitehall, McKnight, and Essel foundations, and USPHS NS-17661,HD-04583, NS-18669,NS-0631, NS-09211, NS-07011, and 5-T32GM07281.

282.3 ULTRASTRUCTURAL EVIDENCE OF CHOLINERGIC SYNAPSES IN DIFFERENT REGIONS OF RAT BRAIN: CHOLINERGIC PATHWAYS V. B.H. Wainer[a], J.P. Bolam*[b], D.J. Clarke*[b], T. Freund*[b], Z. Henderson[b], A.D. Smith[b],and S. Totterdell*[b]. Departments of Pathology and Pediatrics, The University of Chicago, Chicago, IL. 60637, and Departments of Pharmacology and Physiology, University of Oxford, Oxford, OX1 3QT, U.K.

A monoclonal antibody to choline acetyltransferase (Ab8; J. Neurosci.3:1-9, 1983) was used to study the localization of this antigen in rat brain. Fixation was by perfusion with 4% paraformaldehyde, 0.1% glutaraldehyde. Choline acetyltransferase immunoreactivity was localized in vibratome sections (70 microns) by the peroxidase-antiperoxidase method. Sections were then treated with 1% osmium tetroxide and flat-embedded in resin. Light microscopic observation revealed very fine varicose immunoreactive fibres in several brain areas. Parts of these sections were re-embedded so that structures which had been identified in the light microscope could be examined in the electron microscope. Immunoreactive nerve fibres, often containing vesicles, gave rise to synaptic boutons in all the areas so far examined, i.e. cingulate cortex, hippocampus (CA1), basolateral amygdala, neostriatum, and the interpeduncular nucleus. The synaptic boutons had similar ultrastructural features in all areas: they were frequently small and contained large, spherical electron-lucent vesicles. Synapses were of the symmetrical type, although occasional small postsynaptic specializations were observed. Postsynaptic targets were mainly dendritic shafts and spines, and sometimes perikarya. It is concluded that our observations are consistent with acetylcholine being a synaptic transmitter in the rat brain. This work was supported in part by research grants from the Whitehall and McKnight Foundations, USPHS NS-17661 and HD-04583, MRC (U.K.) G979/49, the Wellcome Trust and E.P. Abraham Cephalosporin Trust.

282.4 AN ATLAS OF CHOLINERGIC STRUCTURES IN THE FERRET BRAIN DEMONSTRATED BY IMMUNOCYTOCHEMICAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE: CHOLINERGIC PATHWAYS VII. C. Meininger*,D.B. Rye*, and B.H. Wainer.(SPON: R. Dinerstein). The Dept. of Pathology and Pediatrics, The Univ. of Chicago, Chicago, IL., 60637.

A monoclonal antibody (Ab8, J. Neurosci., 3: 1-9, 1983) against choline acetyltransferase(ChAT) was employed to localize cholinergic structures in the ferret brain using the peroxidase-antiperoxidase method. ChAT immunoreactivity was localized within perikarya of four general groups of nuclei: 1) the striatum, including caudate, putamen, nucleus accumbens, and olfactory tubercle; 2) the basal forebrain, including the nuclei of the diagonal band of Broca, the medial septal nucleus, and the substantia innominata-nucleus basalis of Meynert complex; 3) the mesopontine tegmentum, including the pedunculopontine tegmental nucleus and the lateral dorsal tegmental nucleus; and 4) the cranial nerve motor nuclei.

Fiber staining was observed within major nerve tracts including the fornix, the fasciculus retroflexus, and motor components of the cranial nerves. Collections of fine varicose fibers were observed in many areas of the brain, including cortex, striatum, amygdala, thalamus, hippocampus, and interpeduncular nucleus. The light microscopic appearance of these fibers are what would be expected for preterminal axons and axon terminal fields. The pattern of fine fiber-staining in the caudate-putamen was interesting in that it seemed to be localized in patches rather than uniformly distributed. Similar observations have been made previously for acetylcholinesterase localization(Graybiel and Ragsdale P.N.A.S. 11:5723-5726, 1978). ChAT immunoreactivity was also observed in the medial habenula within fibers and possibly within small perikarya. The resolution of the technique did not permit definitive identification of the latter. The overall distribution and appearance of cholinergic structures in the ferret was similar to what we previously have observed in the rat and monkey. This work was supported by grants from the Whitehall and McKnight foundations, and USPHS NS-17661, HD-04583, and 5-T32GM07281.

282.5 GOLGI - IMPREGNATION OF NEURONS CONTAINING ACETYLCHOLINESTERASE (AChE) OR CHOLINE ACETYLTRANSFERASE (ChAT) IN RAT NEOSTRIATUM: CHOLINERGIC PATHWAYS VI. J. P. Bolam*, C. A. Ingham*, B. H. Wainer and A. D. Smith. Dept. of Pharmacology, University of Oxford U.K. and Dept. Pathology, University of Chicago, U.S.A.

A combination of Golgi-impregnation and acetylcholinesterase (AChE) histochemistry of the neostriatum of DFP-treated rats has identified at least three types of Golgi-stained AChE-positive neurons. Type 1 AChE neurons were large had elongated or spindle shaped perikarya and long tapering dendrites; AChE reaction product was associated with endoplasmic reticulum (ER) nuclear envelope and Golgi apparatus. Type 2 neurons had small to medium sized round or oval perikarya with long essentially smooth dendrites. Type 3 neurons had round large to medium sized perikarya with many smooth frequently branching dendrites. The AChE reaction product of type 2 and 3 neurons was associated with ER and nuclear envelope only. In combined ChAT immunocytochemistry and Golgi-impregnation, so far only type 1 neurons have been both Golgi-stained and immunoreactive.

These observations demonstrate that although there are at least 3 distinct classes of AChE-positive neurons, only one of these is cholinergic.

Supported by grants from The Wellcome Trust, MRC, E.P.A. Cephalosporin Trust, Whitehall Foundation, McKnight Foundation and USPHS grants NS-17661 and HD-04583.

282.6 DEPRESSION AND RECOVERY OF RAT BLOOD AND BRAIN CHOLINESTERASE ACTIVITY AFTER REPEATED EXPOSURE TO SOMAN. J.H. McDonough, T.-M. Shih, A. Kaminskis*, J. Jackson*, and R. Alvarez*. U.S. Army Med. Res. Inst. Chem. Def., Aberdeen Proving Ground, MD 21010.

The purpose of the present study was to determine the magnitude of depression and the time-course of recovery of cholinesterase (ChE) activity following repeated injections of sublethal doses of the potent organophosphorus anticholinesterase compound soman. Male Sprague-Dawley rats were administered 60-45 µg/kg soman or saline subcutaneously 3 times per week for 4 weeks. Groups of animals were killed at 1 hr, 1, 3, 6, 12, and 24 days after the last (12th) injection. Body weight changes were recorded daily throughout the study. ChE activity in plasma, red blood cells (RBC) and six brain regions (brainstem, cortex, hippocampus, midbrain, cerebellum, and striatum) was analyzed by the colorimetric method of Ellman. At 1 hr after the last injection of soman ChE values in all sampled tissues were severely depressed relative to control: RBC=9.6%, plasma=9.8%, brainstem=17.8%, cortex=10.3%, hippocampus=5.8%, midbrain=13.1%, cerebellum=19.0%, striatum=13.9%. At 24 hr after the last injection brain ChE values had approximately doubled over the initial 1 hr values, while RBC values showed no appreciable change and plasma had recovered to 58.9% of control. Recovery of ChE to approximately 50% of control was evident by 6 days in midbrain, brainstem and cerebellum, while RBC, cortex, hippocampus, and striatum ChE levels required 12 days to recover to similar values. Plasma ChE recovered to control levels by 6 days and cerebellum had recovered by 24 days; RBC ChE and ChE in all other brain areas still had not recovered to control levels 24 days after the last injection. Within three injections animals became sensitized to the initial dose of 60 µg/kg soman, requiring first a reduction to 50 µg/kg and then to 45 µg/kg soman over subsequent injections. During the course of this chronic treatment regimen 38% of the subjects died. Body weight loss was found to be the best objective indicator of toxicity. Although some subjects displayed little weight loss in response to a given dose, most subjects, usually in conjunction with the development of noticable symptoms of intoxication, would experience a 5-20 g weight loss. Recovery of this weight loss over the next several days when injections were not given was the best indicator of whether further injections would be well tolerated. As a group, chronically treated subjects had depressed body weight gains relative to controls throughout the chronic dosing period. After the dosing period all groups displayed normal growth throughout the rest of the study. The results indicate that with chronic administration of soman ChE is not depressed uniformly throughout the brain and that recovery of enzymatic function occurs more rapidly in some areas than others.

282.7 BRAIN REGIONAL GLUCOSE USE FOLLOWING EXPOSURE TO SOMAN AND COMPARISON WITH BICUCULLINE AND KAINIC ACID. S.R. Nelson, R. Cross*, M. Giesler*, T. Pazdernik*, K. Mewes*, & F. Samson, University of Kansas Medical Center, Kansas City, Kansas 66103 and J. McDonough, US Army Med. Rsch. Inst. Chemical Def., Aberdeen Proving Ground, MD 21010

The organophosphate, soman is a potent irreversible inhibitor of acetylcholinesterase. At 0.9 LD_{50} doses in rats it induces seizures and causes marked changes in regional glucose use. We compared brain regional changes during seizures induced by bicuculline, kainic acid and soman; also, the effects of soman on brain regional glucose use 1, 2 and 3 days after exposure. Brain regional glucose use was determined by the quantitative (^{14}C)-2-deoxyglucose method (Sokoloff et al. 1977). Bicuculline (0.6 mg/kg; i.v.), a GABA receptor blocker, induces strong, continuous seizures, and marked increases in glucose use in most of the 26 brain regions measured (4 rats). Largest increases were in substantia nigra reticularis (160%) and globus pallidus (180%). The excitotoxin kainic acid (early phase, "wet dog shakes" before clonic/tonic seizure; 6 rats) caused marked increases in septum (434%), dentate gyrus (310%), substantia nigra (77%) and globus pallidus (36%). Bicuculline and kainic acid did not cause as wide spread or as intense an activity as soman. Although similar regions were affected, each convulsant produced a unique pattern. To study the effects of soman 1-3 days after exposure, 17 adult male rats (240-270 g) were given soman (100 µg/kg, i.m., 0.9 LD_{50}), 5 control rats received saline and regional glucose use was measured 24, 48, 72 hours later. Adjacent brain sections were stained with H & E for histology. Almost all 26 brain regions studied were substantially depressed at the three time periods, with 6 regions severely depressed: parietal cortex (-70%), cingulate gyrus (-62%), frontal cortex (-60%), caudate (-58%), ventral thalamus (-58%), medial geniculate (-55%). Glucose use was almost absent in entorhinal cortex, dorsal thalamus and extremely low in frontal cortex (cont.=70, exp.=24 µM/100 g/min.), parietal cortex (cont.=79, exp.=23 µM/100 g/min). We interpret this to indicate impending failure of function leading to neuronal damage. Indeed, extensive pathology occurred in entorhinal and piriform cortex, dorsal thalamus, amygdala and lateral septum. At 72 hours general depression was still profound, and the hippocampal body, dentate gyrus and hilus showed greater depression at 72 hrs than at 24 hrs. We conclude convulsive doses of soman, bicuculline and kainic acid have large effects on brain regional energy flow. Also, the rate of glucose use is a useful indicator of brain pathology and identifies affected regions. We suggest that substantia nigra and related systems are critical in seizure generation. Supported by US Army DAMD-17-78-C-8039.

282.8 TOPOGRAPHICAL DISTRIBUTION OF DECREMENTS IN AND RECOVERY OF MUSCARINIC RECEPTOR BINDING AFTER REPEATED EXPOSURE TO SOMAN. L. Churchill, T.L. Pazdernik*, J.L. Jackson*, S.R. Nelson, & F.E. Samson, Dept. of Anatomy, Pharmacology, and R. L. Smith Res. Ctr., Univ. of KS School of Med., Kansas City, KS, 66103, and J.H. McDonough, Jr., USA Med. Res. Inst. Chem. Def., Aberdeen Proving Ground, MD 21010.

[^{3}H] QNB receptor binding to rat brain muscarinic receptors decreased after repeated exposure to soman, a potent organophosphorus cholinesterase inhibitor. The topographical distribution of this decrement was analyzed using quantitative muscarinic ([^{3}H] QNB) receptor autoradiography. After four weeks of soman exposure (60-45 µg/kg, 3x/wk, s.c.--see abstract, McDonough et al. for details), the [^{3}H] QNB binding decreased to 65-80% of the controls in frontal and parietal cortex, hippocampus, and superior colliculus. Minor or no decreases were observed in caudate putamen, pons, or medial geniculate. Scatchard analyses of saturation curves using frontal cortex sections from soman-treated and control rats revealed a decrease in the maximal binding of [^{3}H] QNB, but no change in affinity. Recovery of [^{3}H] QNB binding to control levels occurred by 12-24 days for the frontal and parietal cortex, hippocampus, and superior colliculus. Analysis of variance and Dunnett's test revealed that statistical differences ($p < 0.05$) between soman-treated and control rats existed for up to 12-24 days after the last soman injection for the above brain regions.

In 15% of the rats treated with this dosage regime of soman, neuropathology was observed in the primary olfactory (piriform) cortex and dorsal thalamus. The ventricles were also abnormally large and the cortex, thinner. Large reductions in [^{3}H] QNB binding and brain regional glucose use (as measured by [^{14}C]-2-deoxyglucose method) appeared in these damaged areas. The decrement and recovery of [^{3}H] QNB binding in undamaged areas of these sections followed the same pattern as found in sections from rats without observable damage. Therefore, the muscarinic receptor binding and rate of glucose use may be useful indicators of brain pathology. Since pathological damage was observed in some soman-treated rat brains, the question arises whether decreases in [^{3}H] QNB binding are due to subtle pathological damage to the cells or due to an adaptive response of the cell to excessive cholinergic stimulation. The recovery of [^{3}H] QNB binding in hippocampus, parietal cortex and superior colliculus (areas without noticeable pathological damage) indicates that decreases in these areas probably reflects adaptive changes. Thanks to C. Beck and K. Mewes for assistance; supported in part by US Army DAMD 17-79-8039.

282.9 EFFECTS OF SOMAN ON LEVELS OF ACETYLCHOLINE AND CHOLINE IN SIX RAT BRAIN AREAS: A DOSE RESPONSE STUDY. T.-M. Shih and T.A. Koviak*, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010.

We have previously reported the time-course effects of a single dose of Soman on levels of acetylcholine (ACh) and choline (Ch) in six discrete rat brain areas (Psychopharmacology, 78:170, 1982). The purpose of the present study was to examine the dose-related effects of Soman on neurotransmitter elevation in these same brain regions. Rats were killed by microwave irradiation focused to the head at 0, 20, 40, 60 and 120 min after a single subcutaneous administration of 0.3, 0.5, 0.7 or 0.9 LD50 (1 LD50= 135 µg/kg) doses of Soman. The levels of ACh and Ch were determined in brainstem (B), cerebral cortex (C), hippocampus (H), midbrain (M), cerebellum (R) and striatum (S) by gas chromatography/mass spectrometry. Data were expressed as percent of control and a comparison was made among these 4 doses of Soman. It was observed that Soman at a dose of 0.5 LD50 or below did not cause appreciable changes of ACh or Ch in any brain area at any time point studied. This is in contrast to our earlier report that Soman produced a dose-related depression on cholinesterase (ChE) and a dose of 0.5 LD50 Soman resulted in approximately a 50% ChE depression in most brain regions (Transact. Am. Soc. Neurochem. 14:148, 1983). Following 0.7 and 0.9 LD50 of Soman, there were progressive increases in both ACh and Ch levels; however, the time to reach maximal levels and the degree and duration of the increase varied in each brain area. After 0.7 LD50, maximal ACh elevation was reached in H, M and B at 40 min (75, 19, and 15%, respectively); in C and R at 60 min (92 and 41%, respectively); in R at 40 min (52%); in M at 60 min (32%); and in C at 120 min (320%). Thus, the greatest degree of change in ACh content after Soman injection occurred in areas C and H. In all brain areas studied maximal elevations of Ch were observed at 40 min and by 60 min had returned to control levels. These data indicate that in contrast of ChE inhibition, ACh and Ch levels were not changed until the dose of Soman was above 0.5 LD50; but similar to the effect on ChE activity, regional differences exist in the degree and duration of ACh or Ch elevation in brain following Soman intoxication. The observed dose-related changes in ACh fit well with other studies indicating that trained or untrained behavior was affected by Soman only at dose above 0.5 LD50.

282.10 EFFECTS OF ATROPINE, PRALIDOXIME AND HI-6 ON SOMAN INDUCED TOXICITY AND ELEVATION OF BRAIN ACETYLCHOLINE AND CHOLINE LEVELS. B.A. Barney* and T.-M. Shih (SPON: R. Ray), US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010.

The purpose of these studies was to examine the effects of atropine sulfate (ATR), pralidoxime chloride (PAM), and HI-6 ([[[(4-aminocarbonyl)pyridino]methoxy]methyl]-2-[(hydroxyimino) methyl]-pyridinium dichloride) on the toxicity and the elevation of acetylcholine (ACh) and choline (Ch) levels in six rat brain areas produced by Soman, a potent cholinesterase inhibitor. Animals were divided into 2 major groups: (I) a pretreatment of ATR (16 mg/kg, i.m.) was given 15 min prior to Soman challenge (100 µg/kg, s.c.), and either PAM (43.16 mg/kg, i.m.) or HI-6 (125 mg/kg, i.p.) was injected immediately after soman: (II) antidotal treatment of ATR or PAM or HI-6 was administered simultaneously with Soman. Rats were killed by microwave irradiation focused to the head thirty min after ATR administration. The levels of ACh and Ch were then analyzed in brainstem, cortex, hippocampus, midbrain, cerebellum and striatum by gas chromatography/mass spectrometry. In separate groups of animals, LD50s of Soman were determined in the absence or presence of ATR, PAM, and HI-6 and the protective ratio, defined as LD50 Soman plus antidote/LD50 soman, was then calculated. It was observed that a single injection of PAM or HI-6 alone had little effect on brain ACh or Ch levels. However, ATR treatment alone reduced ACh in striatum, cortex and hippocampus (38, 25 and 16%, respectively), and increased Ch from 20 to 89% in all brain areas studied. In both pretreatment (I) and treatment (II) groups, neither ATR, PAM or HI-6 showed any effect on Soman induced elevation of ACh except in the striatum, where ACh levels were reduced by ATR, PAM or HI-6 when administered individually. Pretreatment with ATR plus PAM or HI-6 produced an even more significant reduction in striatal ACh levels. The protective ratios of ATR, PAM and HI-6 alone against Soman were found to be 1.2, 1.0, and 2.5, respectively. These data indicate that although ATR, PAM and HI-6 had similar effects upon Soman induced ACh elevation in the different brain areas, HI-6 produced a higher protective ratio than PAM or ATR against Soman intoxication. This protective effect of HI-6 may be due to its peripheral cholinergic or even non-cholinergic central effects, since it was reported earlier that HI-6 does not reactivate brain ChE activities inhibited by Soman in vivo. It appears that neurochemically ATR pretreatment followed by oximes administered therapeutically reduces Soman induced ACh elevation in the brain.

282.11 AN ATLAS OF CHOLINERGIC NEURONS IN THE FOREBRAIN AND UPPER BRAINSTEM OF THE MACAQUE BASED ON MONOCLONAL CHOLINE ACETYLTRANSFERASE IMMUNOHISTOCHEMISTRY: CHOLINERGIC PATHWAYS I. E.J. Mufson, M.M. Mesulam, B.H. Wainer, and A.I. Levey., Harvard Med. Sch., Boston, MA. and University of Chicago, Chicago, ILL.

A monoclonal antibody to choline acetyltransferase (Levey, A.I., Armstrong, D.M., Atweh, S.F., Terry, R.D. and Wainer, B.H., J. Neurosci. 3, 1, 1983) was used to identify the cholinergic cell bodies in the forebrain and upper brainstem of the macaque. Three types of cholinergic neurons were encountered: 1) Intrinsic neurons of the striatum; 2) Motoneurons of the third and fourth cranial nerves; 3) Basal forebrain and pontomesencephalic neurons which have ascending projections to cortex and thalamus,respectively.

The extensive group of basal forebrain cholinergic projection neurons were located in the medial septal region, the diagonal band nuclei and in the nucleus basalis of the substantia innominata. These cholinergic basal forebrain neurons were subdivided into four major groups designated as Ch1-Ch4 (Mesulam M.M., Mufson, E.J., Levey A.I. and Wainer, B.H., J. Comp. Neurol., 214, 170, 1983). The Ch1 cholinergic neurons are the smallest in the Ch groups and they are contained within the medial septal area. The Ch2 neurons are contained within the vertical limb nucleus of the diagonal band. The Ch1 and Ch2 neurons collectively provide the major source of cholinergic projections into the hippocampus. The Ch3 group is contained within the horizontal limb nucleus of the diagonal band and provides cholinergic projections to the olfactory bulb. The Ch4 group is the most extensive and contains the largest neurons. These neurons are found within regions that have variably been called the nucleus basalis, the nucleus of the ansa peduncularis, the nucleus of the ansa lenticularis, and the substantia innominata. The Ch4 group provides the major source of topographically organized cholinergic projections for the entire neocortical mantle.

In the pontomesencephalic region, two groups of cholinergic projection neurons were identified. One group, designated Ch5, is contained mostly within the pedunculopontine nucleus. The other, designated Ch6, is in the region of the laterodorsal tegmental nucleus. The Ch5-Ch6 groups collectively provide the major source of thalamic cholinergic projections and also a minor source of cholinergic innervation into neocortical and hippocampal regions. Supported by the Essel Foundation, the McKnight Foundation, the Whitehall Foundation, and NIH grants NS-02911, NS-07011, NS-17661 and HD-04583.

282.12 NEURAL INPUT INTO THE NUCLEUS BASALIS OF THE SUBSTANTIA INNOMINATA (Ch4) IN RHESUS MONKEY:CHOLINERGIC PATHWAYS II. M.M. Mesulam, and E.J.Mufson. Harvard Med. Sch., Boston, MA.

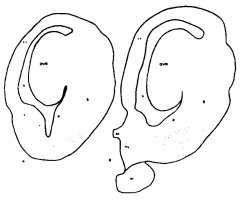
Neurons containing choline acetyltransferase-like immunoreactivity in the nucleus basalis-substantia innominata-ansa peduncularis complex provide the major source of cholinergic innervation for the entire neocortical surface in the monkey brain. On the basis of cytochemistry and connectivity patterns, this group of neurons was designated Ch4 (Mesulam, M.M., Mufson, E.J., Levey A.I. and Wainer, B.H., J. Comp. Neurol., 214, 170, 1983). In 37 rhesus monkeys which had received tritiated amino acid injections in various regions of the brain, we investigated the neural input into Ch4. The results showed that in contrast to their widespread projections to all parts of neocortex, these basal forebrain neurons receive reciprocal projections from only very few cortical areas. Most of the sensory, motor, and association areas in the frontal, parietal, occipital and temporal lobes which are known to receive Ch4 input did not project back to Ch4. The Ch4 neurons received their cortical input from the prepiriform cortex, the orbitofrontal cortex, the anterior insula, the temporal pole, the entorhinal cortex and the medial temporal cortex. There were also subcortical inputs from septal nuclei, the nucleus accumbens-ventral pallidum complex and the hypothalamus. This organization suggests that the Ch4 complex is in a position to act as a cholinergic relay for transmitting predominantly limbic and paralimbic information to the entire neocortical surface. It would also appear that the cortical areas which do not project into Ch4 have no direct way of modulating the cholinergic input which they receive. In contrast, the limited set of cortical areas which do project into Ch4 can influence not only the cholinergic innervation that they receive but also the cholinergic innervation into the entire neocortical mantle. Supported by the Essel Foundation and NIH grants NS-09211 and NS-07011.

282.13 CHOLINERGIC NEURONS IN THE FOREBRAIN OF THE POND TURTLE (PSEUDEMYS SCRIPTA ELEGANS) BASED ON MONOCLONAL CHOLINE ACETYLTRANSFERASE (ChAT) IMMUNOHISTOCHEMISTRY: CHOLINERGIC PATHWAYS III. P.H. Desan, E.J. Mufson, M.M. Mesulam, B.H. Wainer and A.I. Levey, Harvard Med. Sch., Boston, MA. and University of Chicago, Chicago, ILL.

Cholinergic neurons in the basal forebrain of the pond turtle were examined using a monoclonal antibody to choline acetyltransferase (Levey, A.I., Armstrong, D.M., Atweh, S.F., Terry, R.D. and Wainer, B.H. J. Neurosci., 3:1, 1983). This antibody, raised against mammalian ChAT, also gave a positive immunohistochemical reaction in neurons of the turtle brain presumed to be cholinergic such as those in brain stem motor nuclei and spinal cord ventral horn. Control sections reacted with irrelevant IgG instead of the ChAT antibody showed no neuronal staining.

Neurons containing ChAT-like immunoreactivity occupied a continuous zone of the basal telecephalon extending from the level of the olfactory turbercle to the level of the amygdaloid region (see Figure). Numerous cholinergic neurons were scattered among the fibers of the medial and lateral forebrain bundles in a position ventral to areas c, d and 9 (Riss, W., Halpern, M. and Scalia, F., Brain Behav. Evol., 2:1, 1968) which together have been considered the homologue of the basal ganglia of mammals. Horseradish peroxidase injections in the dorsal or medial cortex of the turtle revealed labeled neurons within the region which contained these cholinergic neurons, suggesting that the ChAT-positive neurons of the forebrain may provide cholinergic projections to the cortical mantle.

In the mammalian telencephalon, cholinergic cell bodies are found in the striatum and in the basal forebrain. In the basal forebrain the cholinergic neurons are located in the septum, in the diagonal band nuclei and in the nucleus basalis of the substantia innominata. These basal forebrain neurons which provide the major source of cholinergic projection for telencephalic structures can be subdivided into four major groups (Ch1-Ch4) on the basis of their cytochemical and connectivity patterns (Mesulam, M.M., Mufson, E.J., Levey, A.I. and Wainer, B.H., J. Comp. Neurol., 214, 170, 1983). Perhaps the cholinergic neurons of the turtle forebrain collectively constitute a forerunner for the Ch1-Ch4 neurons and even for the cholinergic striatal neurons. These neurons may become progressively more differentiated into separate groups in the course of phylogenetic evolution. Abbrev:ac-anterior commissure, cx-cortex; DVR-dorsal ventricular ridge; hy-hypothalamus; oc-optic chiasma; s-septum; v-ventricle. Support:Essel,McKnight, Whitehall Found.,NS02911,17661,07011.



282.14 THE EFFECT OF SELECTIVE LESIONS ON VESTIBULAR NUCLEAR COMPLEX (VNC) CHOLINE ACETYLTRANSFERASE (CAT) ACTIVITY IN THE RAT. Robert E. Burke, S. Fahn, Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, New York

Although central vestibular systems play an important role in the physiology of motor control, little is known about their neurochemical basis. Acetylcholine (ACh) has been thought to play a functional role in VNC because it excites vestibular cells, and because muscarinic cholinergic receptors are present in VNC. In addition, we have found that VNC microinjections of cholinergic antagonists have a marked influence on postural control in awake rats, producing postural changes identical to those induced by labyrinthectomy.

Although ACh may play a role in central vestibular function, the source(s) of cholinergic input to VNC is at present unknown. This study sought to clarify cholinergic VNC anatomy in rat by measuring CAT activity following lesions of intrinsic neurons or of known vestibular afferents. VNC was dissected from the dorsolateral portion of a standarized hindbrain coronal section, obtained by use of a plexiglass brain well. VNC CAT activity (50± 5 pmol/hr/ug protein) was less than that of striatum (200±16), hippocampus (65±7) and frontal cortex (80±8), but an order of magnitude greater than that of cerebellum (4±0.6). Kainate (0.75 ug/0.5 uL) injected into VNC was without effect on CAT activity (ipsilateral 99±8% of contralateral, n=9) at the tenth postoperative day. Section of cranial n. VIII did not decrease ipsilateral CAT activity (ipsilateral 132±15% of contralateral, n=6); the apparent increase seemed to be due to a lower protein content on the operated side. Cerebellectomy (93±6%, n=13; sham controls 100±6%, n=14), vestibular commissurotomy (96±10%, n=6; sham controls 100±8%, n=8) and T3 cordotomy (95±4%, n=8; sham controls 100±8%, n=7) were without significant effect on VNC CAT activity. We conclude that most VNC CAT activity is not localized to intrinsic neurons or afferents from Scarpa's ganglion, cerebellum, the contralateral VNC, or cord. It is possible that cholinergic projections arise from reticular nuclei or from rostral structures.

Supported by the Dystonia Medical Research Foundation.

282.15 RETROGRADE TRANSPORT OF [^{3}H]CHOLINE FOLLOWING INJECTION IN THE VAGAL COMPLEX S.L. Morzorati, J.R. Simon, M.H. Aprison, Inst. of Psychiatric Research and Depts. of Psychiat. and Biochem., Indiana Univ. Sch. of Medicine, Indianapolis, IN 46223.

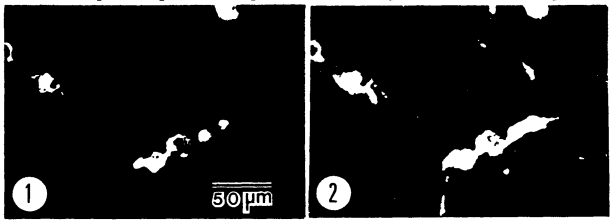
The retrograde transport of transmitters or precursors has been used to autoradiographically label specific neuronal pathways in a number of species. Thus, the septohippocampal pathway of the rat, a known cholinergic pathway, was selectively labeled after an injection of [^{3}H]choline into the hippocampus. More recently, transmitter-specific retrograde labeling of neurons has been utilized to identify cholinergic projections not yet described. An injection of [^{3}H]choline in the cervical cord of the rat led to the supposition by Pare et al. (1982) that certain cholinergic brainstem neurons project to this area. Biochemical studies of cholinergic parameters in the caudal medulla of the rat suggested the presence of a substantial cholinergic input to the vagal complex (Simon et al., Neurochem. Res. 6:497 1981). Preliminary studies in our laboratory employing choline uptake following hemisections placed at various anterior-posterior planes indicated that this cholinergic input originates rostral to the colliculi but caudal to the anterior hypothalamus. In the present study, the technique of retrograde transport of choline was applied to determine more precisely the location of these cholinergic neurons which project to the medulla. Male rats were anesthetized with Pentobarbital and [^{3}H]choline (3 uCi in 20 nl) was injected unilaterally through a glass micropipette into the vagal complex. The animals were sacrificed by decapitation at various times (2-72 hrs) following the injection. The brainstem was isolated from the forebrain by a cut made just caudal to the colliculi. The brainstem and forebrain were then frozen separately on microtome chucks. The medulla was cut into 25 μm sections on a Cryostat and histologically prepared in order to locate the site of injection. The forebrain was cut into 500 μm samples, each sample was placed in a counting vial and prepared for liquid scintillation spectroscopy. The radioactivity in each sample was plotted against the distance of that sample from the interaural line in order to map the rostrocaudal accumulation of radioactivity in the forebrain. A peak of radioactivity was consistently seen in the forebrain following injections of [^{3}H]choline in the vagal complex. This peak was evident some 7-9 mm rostral to the interaural line. Considering that retrograde transport proceeds at a rate of 1 to 6 mm/hour, the observed peak is consistent with the operation of a retorgrade transport mechanism. The ramifications of these results will be discussed. (Supported by NIH Grant NS 16205).

282.16 CHOLINE ACETYLTRANSFERASE-CONTAINING NEURONS IN THE PRIMATE BASAL FOREBRAIN AND STRIATUM: IMMUNOCYTOCHEMICAL STUDIES. C. A. Kitt†, J. C. Hedreen†, S. J. Bacon†*, M. W. Bechert*, P. M. Salvaterra††, A. I. Levey†††, B. H. Wainer††† and D. L. Price†. †Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205; ††Div. of Neuroscience, City of Hope Res. Inst., Duarte, CA 91010; †††Dept. of Pathology, Univ. of Chicago, Chicago, IL 60637.

The distribution of cholinergic neurons in the forebrain of monkeys was investigated by immunocytochemical methods using two different anti-choline acetyltransferase (ChAT) monoclonal antibodies (Levey, A.I. et al., Brain. Res., 218:383-387, 1981; Crawford, G.D., Correa, L. and Salvaterra, P.M., Proc. Natl. Acad. Sci. USA, 79:7031-7040, 1982). Both antibodies showed ChAT immunoreactivity in neurons within the medial septum, nucleus of the diagonal band of Broca (ndbB) (Ch2), nucleus basalis of Meynert (nbM) (Ch4), and neostriatum; control sections showed no immunoreactivity in these cell groups. In the striatum, scattered large neurons showed ChAT immunoreactivity. A large group of ChAT-immunoreactive perikarya was seen in the ndbB, which was continuous caudolaterally with the large group of ChAT-immunoreactive nbM neurons within the substantia innominata, ventral to the globus pallidus. Caudal to the major cluster of neurons in the nbM, ChAT-positive nerve cells were consistently seen in large numbers ventral to the globus pallidus. Large, ChAT-immunoreactive neurons were also seen in the precommissural fornix, caudolateral septum, white matter underlying the nucleus accumbens and putamen, clustered along the edges of the anterior commissure in the lateral and medial medullary laminae of the globus pallidus, and scattered within and at the medial edge of the internal capsule. This distribution of ChAT-immunoreactive neurons parallels that of the large chromophilic neurons identified in Nissl preparations and acetylcholinesterase-positive neurons seen with histochemical methods. Dysfunction and death of cholinergic neurons in human basal forebrain may be the pathological substrate of the cholinergic deficit occurring in the cortex in patients with Alzheimer's disease.

282.PO **CHOLINERGIC PROJECTIONS REVEALED BY CHOLINE ACETYLTRANSFERASE (ChAT) IMMUNOHISTOCHEMISTRY AND FLUORESCENT TRACER HISTOLOGY PERFORMED ON THE SAME TISSUE SECTION.** N. J. Woolf, F. Eckenstein* and L. L. Butcher. Dept. of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024, USA and *Max-Planck-Institut für Psychiatrie, Abteilung Neurochemie, Am Klopferspitz, D-8033 Martinsried, FRG.

Putative cholinergic projections to the frontal cortex, basolateral amygdala, and nucleus tegmenti pedunculo pontis were traced by infusing propidium iodide (PI), a fluorescent marker, into those sructures and processing the same tissue sections immunohistochemically for ChAT by use of both polyclonal and monoclonal antibodies [Eckenstein and Thoenen, 1982, EMBO J., 1:363-368]. Following tracer infusions into the frontal cortex, PI-labelled cells (Fig. 1) that also demonstrated ChAT-like immunoreactivity (FITC label, Fig. 2) were found in the nucleus basalis (Figs. 1 & 2) and the magnocellular preoptic area [terminology: Bigl et al., 1982, Brain Res. Bull., 8:727-749]. Although the nucleus basalis and magnocellular preoptic area in these brains contained many other ChAT-positive cells that did not transport the tracer, virtually all PI-labelled cells in those regions were also immunoreactive for ChAT. When the tracer was infused into the basolateral amygdala, PI-labelled cells were found in the nucleus of the diagonal band, the ventral pallidum/lateral preoptic area, the magnocellular preoptic area, the nucleus basalis, and the nucleus of the ansa lenticularis. Although many of these PI-labelled cells also showed ChAT-like immunoreactivity, some did not. After the tracer was infused into the nucleus tegmenti pedunculo pontis many basal forebrain neurons transported the marker that were not ChAT-positive but were located in regions containing many ChAT-positive cells. ChAT-positive cells of the contalateral nucleus tegmenti pedunculo pontis did, however, transport PI. The simultaneous demonstration of ChAT and fluorescent tracers provides the most direct evidence for cholinergic pathways from the basal forebrain to the cortex and amygdala, and from the contralateral nucleus tegmenti pedunculo pontis to its ipsilateral counterpart.



ACETYLCHOLINE: BIOSYNTHESIS AND ELECTROPHYSIOLOGY

283.1 DI-ISOPROPYLFLUOROPHOSPHATE INDUCED INCREASE IN UNIT ACTIVITY IN THE RAT SUPERIOR COLLICULUS: DOSE-RESPONSE, SITES OF ACTION AND CHOLINERGIC RECEPTORS. P.D. Cheney and R.J. Kasser*. (SPON: S. Nelson). Departments of Physiology and Anatomy, University of Kansas Medical Center, Kansas City, KS. 66103.

Using the 2-deoxyglucose technique, Nelson et al. (Brain Res. 157:186, 1980) reported that systemic injection of the acetylcholinesterase inhibitor di-isopropylfluorophosphate (DFP) increased glucose utilization in the stratum griseum superficiale (SGS) of the superior colliculus. Although this finding suggests an increase in neuronal electrical activity, additional factors can also increase glucose utilization. The purpose of this study was to: 1) detail the changes in spontaneous and light evoked SGS unit activity associated with DFP treatment, and 2) determine the sites of action of DFP in the retino-tectal visual system and the cholinergic receptor types involved. Albino rats weighing 250-475g were anesthetized with halothane, and a tungsten microelectrode was stereotaxically positioned in the SGS. The SGS was identified by its high spontaneous activity and its distinctive transient responses to the onset and offset of a diffuse light stimulus. Systemic injection of DFP produced a dose dependent increase in SGS spontaneous multiunit activity and a reduction in light evoked activity over a dose range from 0.5 to 2.0 mg/kg. The reversal time for these effects, following systemic injection of 1.3 mg/kg DFP was about 4.5 hours.

To determine whether DFP acts at: 1) retinal sites, we injected DFP intraocularly (1.5 ug in saline) while recording SGS unit activity, 2) sites within the CNS, we injected DFP systemically (1.3 mg/kg) in bilateral enucleate animals. Significant increases in spontaneous multiunit activity occurred in both groups suggesting that DFP's action on the SGS involves both retinal and central synapses. Are the actions of DFP mediated by muscarinic or nicotinic cholinergic receptors? Pretreatment with atropine (a muscarinic antagonist), administered by the same route as DFP, completely blocked the DFP induced increase in SGS unit activity described above. However, mecamylamine (a nicotinic antagonist) pretreatment was ineffective. Furthermore, oxotremorine (a muscarinic agonist) administered intraocularly or systemically in intact or bilateral enucleate animals produced increases in SGS unit activity similar to those obtained with DFP. Oxotremorine evoked increases in SGS unit activity were fully blocked by either atropine or scopolamine. We conclude that DFP acts at muscarinic synapses both centrally and in the retina to increase unit activity in the SGS of the superior colliculus. Neither atropine nor mecamylamine reduced light evoked activity in the SGS suggesting that these responses of the retino-tectal pathway are not dependent on cholinergic synapses. Nevertheless, the cholinergic system is capable of a powerful excitatory action on SGS and may function to modulate the background firing rate of SGS neurons.

283.2 RESPONSE OF RAT ISOLATED PHRENIC NERVE HEMIDIAPHRAGM TO DFP OR SOMAN AND HC-3 REVERSAL. P.A. Grieve* and W.G. VanMeter. Dept. Veterinary Physiology and Pharmacology, Coll. Vet. Med., Iowa State University, Ames, Iowa 50011.

Single twitch and tetanic contractions of the rat isolated phrenic nerve hemidiaphragm have been studied after exposure to DFP in vitro or SOMAN in vivo with antagonism of these responses by subsequent in vitro application of hemicholinium 3 (HC-3).

Due to the toxicity of SOMAN subcutaneous injections of 1, 2 and 4X the LD50 (LD50 = 80mcgm/kg) were given to female Sprague-Dawley or Sprague-Dawley/Norwegian cross-bred rats. Thirty minutes post injection (or on death) the left hemidiaphragm phrenic nerve was placed in a 50ml isolated tissue bath, with Krebs solution at 37°C and aerated with 95% O_2 5% CO_2. Cathodal, 1Hz, 1msec supramaximal stimuli were applied to the phrenic nerve for evoking single twitch responses and 50Hz for 5sec was used to evoke tetanic contractions. For DFP studies, rats were killed and the left hemidiaphragm phrenic nerve was prepared as described above prior to in vitro application of 1.0 or 2.0mcgm/ml DFP. Single twitch responses were unaffected by 1 or 2X LD50 SOMAN and were initially depressed for 30 minutes in rats receiving 4X LD50. Tetanic contractions were markedly depressed in 75% (N=8) of rats receiving 2 or 4X LD50 SOMAN. Post tetanic potentiation (PTP) was initially absent and a marked post tetanic depression (PTD) was seen. Both the inhibition of 50Hz tetanic contraction and the PTD were antagonised by HC-3 (40mcgm/ml) and the PTD was reversed to PTP. Tetanic contractions in rats receiving 1X LD50 SOMAN were indistinguishable from controls.

DFP had little to no effect on the amplitude of single twitch responses. Tetanic contractions were markedly depressed with 1.0 or 2.0mcgm/ml DFP. While PTD was less pronounced compared to the effect of SOMAN it was readily antagonised and reversed by 40mcgm/ml HC-3. Tetanic contractions were restored in presence of HC-3.

Work supported by U.S. Department of Defense Contract. Nr.DAMD17-80-C-0106

283.3 SEPTO-HIPPOCAMPAL MODULATION: INTRACELLULAR OBSERVATIONS IN SITU. N. Ropert and K. Krnjević. Departments of Physiology and Anaesthesia Research, McGill University, Montréal, Canada.

According to previous extracellular observations (Krnjević & Ropert, Neuroscience, 1982, 7:2165) electrical stimulation of the medial septum that elicits minimal field responses in the CA1-CA3 region of the hippocampus nevertheless strongly facilitates population spikes evoked by commissural stimulation. The pharmacological characteristics of this effect indicate a cholinergic mechanism of action; but its rapid time course is more consistent with a presynaptic disinhibitory effect of ACh (Ben-Ari et al., Neuroscience, 1981, 6:2475) than with a post-synaptic reduction of K currents, which typically has a very slow time course. In the present experiments, we have recorded intracellularly from CA1, CA2 and CA3 neurons in rats under urethane anaesthesia. Bipolar stimulating electrodes were inserted stereotaxically into the commissure and the medial septum. Positioning of the septal stimulating electrodes was considered correct when single shocks or brief tetani (typically 10 pulses at 50-100 Hz), which by themselves produced only minimal fields in the hippocampus, clearly facilitated population spikes. Septal stimulation was tested at several intensities to establish the threshold for the septal modulatory action.

As in previous experiments on the hippocampus in situ, intracellular impalements of CA2/3 and especially CA1 neurons were of variable but mostly short duration. Nevertheless, all neurons showed typical IPSPs and corresponding increases in input conductance in response to commissural stimulation. The most consistent effect of brief septal tetani (preceding single commissural shocks by 20-30 ms) was a reduction of the evoked IPSP conductance increase - which affected especially the latter phase of the IPSP - and also a marked reduction in tonic inhibitory drive. These effects were clearly related to the intensity of septal stimulation, being obtained only when the latter was suprathreshold for the facilitation of population spikes. They were quick in onset (within 10s), and equally quickly reversible. When recording conditions were particularly stable, a depression of IPSPs could be reproduced several times. Most often, there was a tendency to depolarization, but changes in resting input resistance were quite variable with some increase being observed more often than a decrease. In conclusion, these intracellular observations are consistent with the idea that cholinergic disinhibition of pyramidal cells is an important mechanism of septo-hippocampal facilitation.

Supported by the Canadian Medical Research Council.

283.4 EFFECTS OF CHOLINESTERASE INHIBITORS AND ANTIDOTES ON EXTRARETINAL PHOTORECEPTORS IN APLYSIA. J.P. Apland. Sensory Research Division, U.S. Army Aeromedical Research Laboratory, Fort Rucker, AL 36362.

The effects of cholinesterase inhibitors and antidotes on extraretinal photoreceptor cells in Aplysia were investigated with electrophysiological methods. The identified extraretinal cells R_2, pleural giant, and ventral photoresponsive neuron (v.p.n.) have been shown to hyperpolarize in response to light (Andresen and Brown, J. Physiol. 287: 267, 1979). The process of light transduction in these three cells is similar to that proposed in the calcium hypothesis for vertebrate rod outer segments (Hagins, Ann. Rev. Biophys. Bioeng. 1: 131, 1972). Diisopropylfluorophosphate (DFP), which is a potent, irreversible organophosphate cholinesterase inhibitor, consistently attenuated the photoresponse elicited by flashes of light when superfused on all three cells. Attenuation of photoresponse was dose-dependent, with 50% attenuation in all cells at a DFP concentration of 10^{-3} M. Physostigmine, a reversible carbamate-type cholinesterase inhibitor, also attenuated the photoresponse in a dose-dependent manner. Physostigmine was, however, less potent, with 50% attenuation occurring at a concentration of 5×10^{-3} M. Photoresponse attenuation with both drugs was completely reversible upon washout of drug. d-tubocurarine, which blocks nicotinic acetylcholine (ACh) receptors, had no effect on the photoresponse by itself, and did not prevent the attenuation of photoresponse caused by DFP. The muscarinic ACh receptor antagonist, atropine, also had no effect by itself on the photoresponse in these cells. However, atropine (10^{-3} M) did block the attenuation of photoresponse caused by DFP. DFP, physostigmine, and atropine all caused depolarization of the resting membrane potential in extraretinal photoreceptor cells, whereas d-tubocurarine elicited a significant hyperpolarization. Neither diazepam nor the oxime, 2-PAM, which is a cholinesterase reactivator, had any effect on the photoresponse. Neither drug blocked the attenuation of photoresponse caused by DFP.

The reversibility of photoresponse attenuation upon washout of DFP suggests that this drug is acting in some manner other than by cholinesterase inhibition. However, the block of DFP's effect by atropine suggests that ACh may be involved in some manner.

283.5 MECHANISMS OF FACILITATION OF ANTIDROMIC POPULATION SPIKES BY IONTOPHORETIC APPLICATIONS OF ACETYLCHOLINE INTO HIPPOCAMPAL CA3 REGION, IN SITU. T. Dalkara*, N. Ropert, C.Y. Yim* and K. Krnjević. Department of Anaesthesia Research, McGill University, Montréal, Québec, Canada.

As already reported (Dalkara et al., Proc. Can. Fed. Biol. Soc. 1983, in press) a curious feature of the CA3 population spike evoked antidromically (peak latency = 2 ms) by fimbrial stimulation is that it can often be markedly potentiated by microiontophoretic release of acetylcholine (ACh) at the site of recording in the CA3 pyramidal layer. Two possible explanations for this phenomenon are as follows: 1. Impulses travelling along CA3 efferent axons fail to invade a certain proportion of CA3 cell bodies, because of an unfavourable geometry, perhaps aggravated by a high level of tonic inhibition; ACh would make antidromic invasions possible by raising the excitability of the soma. 2. The subliminal excitatory action of ACh facilitates the excitation of quiescent cells by electrical fields generated by neighbouring active cells. To distinguish between these two hypotheses, ACh was tested systematically in combination with a wide range of fimbrial stimulation, from threshold to maximal. If the first hypothesis is correct one would expect ACh to cause an approximately constant proportional increase in the CA3 population spike over the full range of fimbrial stimulation intensity. If the second is correct, ACh should have a minimal or no effect at low and at high stimulation intensities (in the first case, because the extracellular field is insufficient to produce excitation, even in the presence of ACh; in the second case, because there are no remaining quiescent cells to be excited), but it should be particularly effective with near half-maximal stimulation when the extracellular field is relatively large, and there are still many cells to be excited.

In rats under urethane anaesthesia, ACh tests have consistently shown little or no enhancement of antidromic population spikes when the response was <20% or >90% of maximum; and a variable increase, reaching a peak at around 40% of maximum. Simultaneous recordings of the CA3 population spike and the axonal compound action potential in the contralateral hippocampal commissure showed no change in the latter when the CA3 antidromic response was enhanced by ACh, thus indicating no increase in excitability of fimbrial fibres. These observations provide further support for the evidence that ephaptic currents are a significant factor in promoting synchronized firing of hippocampal pyramidal neurones both in vitro (Taylor & Dudek, 1982, Science, 218, 810) and in situ (Taylor, Krnjević & Ropert, 1982, Can. Physiology, 13, 143).

With financial support from MRC of Canada.

283.6 PROPERTIES OF SEPTO-HIPPOCAMPAL NEURONS IDENTIFIED BY ANTIDROMIC STIMULATION. Y. Lamour, P. Dutar* and A. Jobert*, Unité de Recherches de Neurophysiologie Pharmacologique, INSERM, U. 161, 2 rue d'Alésia, 75014 Paris (France).

Septo-hippocampal neurons (SHNs) were recorded in the medial septum-diagonal band (vertical limb) area in rats anaesthetized with urethane (1.5 g/kg i.p.). SHNs were identified by their antidromic response following electrical stimulation of the fimbria. Criteria for antidromic invasion were : collision with orthodromic spikes, fixed latency and ability to follow high rates of stimulation (> 250 Hz). The pharmacological properties of these identified SHNs were studied using microiontophoretic applications from multibarreled electrodes filled with cholinergic agonists and antagonists. The position of the SHNs was marked by a local release of dye which was subsequently located on brain sections. SHNs (n = 118) were located in the medial septum-diagonal band area, close to the midline. Spontaneous activity of SHNs was relatively high, 70% of them having a discharge rate higher than 10 impulses per second. 65% of the SHNs discharged rhythmically in characteristic bursts with a frequency of 3 to 5 bursts per second. In some neurons, rhythmic firing alternated with period of more regular firing. The mean latency of antidromic activation was 2.2 ms (range 0.6 - 9 ms), which gives an average conduction velocity of about 1.5 m/s. Most of the SHNs were also inhibited by fimbria stimulation at intensities lower than the threshold for antidromic activation. A large percentage of SHNs could be excited by acetylcholine (77%) and carbachol (72%). Other cholinergic agonists were not as effective. Responses to carbachol were of larger amplitude and longer duration than those to ACh. Responses to ACh could be abolished by a simultaneous application of atropine. Our results reveal that i) SHNs are located in the medial septum-diagonal band area, in agreement with anatomical observations ii) many of these SHNs display a rhythmic mode of spontaneous discharge at about 4 bursts per second iii) most of them are excited by acetylcholine and carbachol, applied by iontophoresis. Numerous experimental data point to the cholinergic nature of the septo-hippocampal pathway. We assume that at least some of the SHNs identified by antidromic stimulation in our study are cholinergic. It follows that cholinergic septo-hippocampal neurons can be excited by acetylcholine itself.

283.7 DE NOVO SYNTHESES OF CHOLINE IN CRUDE SYNAPTOSOMES FROM RAT'S BRAIN IS NOT INFLUENCED BY ATROPINE OR HEMICOLINIUM-3. C.F. Leprohon and R.J. Wurtman (Spon: G. H. Anderson). Laboratory of Neuroendocrine Regulation. MIT. Cambridge, MA 02139.

Mammalian neurons synthesize choline de novo by methylating phosphatidylethanolamine (PE) and hydrolyzing the resulting phosphatidylcholine (PC). The methylation reaction is catalyzed by the enzyme complex phosphatidylethanolamine-N-methyltransferase (PeMT) and uses S-adenosylmethionine (SAM) as a methyl donor. Since the liberated choline produced from phospholipid methylation may be available for acetylcholine biosynthesis, we examined whether pharmacologic manipulations which increase the cholinergic neuron's need for free choline would affect the rate of choline production in rat brain's synaptosomal membranes.

Synaptosomes were resuspended in buffer (pH 7.4) containing 2.5 uCi ^{3}H-SAM (15 Ci/mmol) and atropine (10^{-9} to 10^{-4} M). After 30 minutes of incubation, atropine had no effect on the rate of PE methylation as measured by the incorporation of ^{3}H-CH_3 groups into PE.

To determine if more prolonged attenuation of cholinergic activity would influence PeMT activity, rats were injected with atropine (i.p., 25 mg/kg) and hemicolinium-3 (i.vt., 10 ug) every 4 hrs for 24 hrs. Brain acetylcholine concentrations in cortex, hippocampus and striatum were depleted by 50%, 40% and 70%, respectively. Once again, PeMT activity was similar in the synaptosome-enriched P2 pellets from these three regions.

The results of these studies indicate that short term (up to 24 hours) enhancement of cholinergic activity does not affect the rate of PE methylation in synaptosomal membranes. However, several factors may be responsible for a lack of effect of atropine and hemicolinium-3 on PeMT activity. First, this was a relatively short term experiment, and enzyme activation may require additional time in order to be measurable. Second, if preexisting PC was being hydrolyzed to yield free choline, perhaps PeMT is sensitive to changes in membranal PC concentration and PC hydrolyses had not proceeded to such an extent as to stimulate PeMT activity. Third, since cholinergic synaptosomes could not be isolated, perhaps PeMT was activated in cholinergic cells but this effect was diluted out by measuring PeMT activity in a mixed synaptosomal preparation. Finally, the methylation pathway may be important for maintaining normal cholinergic tone, but may not function as an emergency pathway to provide additional choline on a short term basis. Experimental models other than the In vitro incubation of rat's brain synaptosomes, may provide more useful information to assess the importance of PE methylation in providing choline for acetylcholine biosynthesis. (CEL is a Canadian MRC Fellow, supported by grant MH-28783).

283.8 SOURCE OF CHOLINE FOR THE RELEASE OF CHOLINE AND ACETYLCHOLINE FROM BRAIN SLICES J.C. Maire*, M.T. Tacconi* and R.J. Wurtman (SPON: P.B. Dews) Lab. of Neuroendocrine Regulation, MIT, Cambridge, MA 02139.

Cholinergic neurons are dependent upon choline for the synthesis of the transmitter, acetylcholine (ACh). The intracellular level of choline can modulate the rate at which ACh is synthesized and released. In situations when ACh release is increased, it is not clearly established whether some choline-containing compounds in the neuron (such as phosphocholine or phosphatidylcholine) can constitute a source of choline for ACh biosynthesis.

Slices from rat striatum were superfused with physiological solution (PS). After a period of equilibration, choline and ACh were measured in the effluent. Slices were removed from the apparatus at the beginning and at the end of the collection period, and the tissue choline and ACh contents were measured.

After a 1 hr superfusion period in choline-free PS containing 20 µM eserine, the mean rate of appearance of choline and ACh in the effluent were 60 and 7.5 pmole.min^{-1}.mg prot^{-1} respectively. During the 40 min collection period, the tissue contents varied from 2.2 to 2.3 nmole. mg prot^{-1} for choline, and from 2.6 to 2.4 nmole.mg prot^{-1} for ACh. Electrical stimulation of the slices led to a transient increase in the liberation of ACh, but not of choline. When hemicholinium, an inhibitor of the high affinity uptake of choline, was added to the PS, there was a 1.75 fold increase in the rate of choline efflux, while ACh release was reduced by half. The decrease in choline and ACh in the tissue during that experiment has been found to account for 75% of the release of these substances.

Futhermore, the appearance of free-fatty acids (FFA) in the effluent has been measured in a set of similar experiments conducted in PS containing defatted albumin. The rate of FFA release reached a constant of 350 pmole.min^{-1}.mg prot^{-1} 90 min after the beginning of the superfusion. The efflux of FFA was not altered by the presence of hemicholinium in the PS, and was slightly and transiently decreased during electrical stimulation.

In all these experiments, the changes in the levels of choline and ACh in the tissue cannot account for the total release of these substances into the medium. Some other choline-containing compounds must be involved. In absence of exogenous choline in the PS, the observed release of FFA could indicate a contribution of the choline-containing phospholipids to the supply of choline. [J.C.M. holds a fellowship from the Swiss National Science Foundation; M.T.T. holds a fellowship from the Center for Brain Science and Metabolism; these studies were supported in part by an NIMH grant (MH-28783).]

283.9 DIFFERENTIAL REGULATION OF AGONIST BINDING TO PUTATIVE SUBTYPES OF THE MUSCARINIC RECEPTOR. Thomas W. Vickroy, William R. Roeske* and Henry I Yamamura. Depts. of Pharamcology, Biochemistry and Internal Medicine, Univ. of Arizona, Tucson, AZ 85724.

Previous physiological and biochemical studies have indicated that functionally distinct subtypes of the muscarinic receptor (classified as M_1 and M_2) may be present in various mammalian tissues. While the concept of M_1 and M_2 receptor subtypes is primarily based upon the selective physiological actions and binding properties of the nonclassical muscarinic antagonist pirenzepine, little is known of the agonist binding properties of these muscarinic receptor subtypes. In the present studies, we have directly studied agonist binding to membranes from tissues which purportedly contain M_1 and/or M_2 receptor sites. As discussed below, the regulatory influences of several agents upon agonist binding differs between the muscarinic receptor subtypes.

High-affinity muscarinic agonist binding was measured in homogenates of rat cerebral cortex, cerebellum and whole heart with the muscarinic agonist [^{3}H]cis methyldioxolane ([^{3}H]CD) using a recently developed rapid filtration binding assay. Nonspecific [^{3}H]CD binding, as defined with a 1µM atropine sulfate, (less than 10% of total binding), was determined under all experimental conditions.

In the heart and cerebellum, tissues which contain predominantly M_2 sites, the guanine triphosphate analogue Gpp(NH)p (guanyl-5'-yl imidodiphosphate) is a potent inhibitor of [^{3}H]CD binding (IC_{50}=2µM). Conversely, in the cerebral cortex, a tissue containing mostly M_1 sites, Gpp(NH)p had no significant effect on high-affinity [^{3}H]CD binding at concentrations up to 100 µM. In addition to these differential effects of Gpp(NH)p, magnesium ions also produced selective effects. In the M_2 tissues (heart and cerebellum), low concentrations of magnesium ions (less than 1mM) enhanced [^{3}H]CD binding by as much as 150 percent whereas higher magnesium concentrations significantly reduced binding. In the M_1 tissue (cerebral cortex), magnesium had few detectable effects on high-affinity [^{3}H]CD binding. Finally the effects of NEM (N-ethylmaleimide), a sulfhydryl alkylating agent, was compared in these tissues. As observed with guanine nucleotides and magnesium ions, the effect of NEM in M_2 tissues (diminished [^{3}H]CD binding) differed from its effect in M_1 tissues (increased [^{3}H]CD binding). Preliminary kinetic analyses of certain binding alterations support a model of allosterically-induced changes in binding sites affinities.

In conclusion, it appears that agonist binding to putative M_1 and M_2 muscarinic receptor subtypes is differentially affected by guanine nucleotides, magnesium ions and NEM. The mechanistic nature and potential significance of these distinct effects are under further investigation. (Supported by USPHS grants)

283.10 MUSCARINIC AUTORECEPTORS AND ACH RELEASE. E.M. Meyer and E. St. Onge*. Department of Pharmacology and Therapeutics, U. of Florida, Gainesville, FL 32610.

Presynaptic muscarinic autoreceptors fine tune transmission by attenuating acetylcholine (ACh) release. The mechanism underlying this release modulation is obscure, but one likely site of action is at the level of voltage-dependent calcium entry: the trigger for neurotransmitter release. We therefore studied the possible modulatory role of this calcium uptake by measuring nerve terminal ACh release under basal conditions as well as in the presence of several secretogogues that elevate intracellular calcium levels through different membrane processes.

Cerebral cortical synaptosomes from male Sprague Dawley albino rats were preincubated with (^{3}H)-choline to load them with (^{3}H)-ACh, washed, and then incubated at 37^c for 30 s in Krebs Ringer buffer (KR) with 10 µM eserine sulfate, ± 1 mM calcium, ± 1 mM EGTA, ± 10 µM oxotremorine, ± 100 µM carbachol, and ± 1 µM atropine. Next, the calcium ionophore A23187 (0-10 µg/ml), KCl (5.5-60 mM), or ouabain (0-500 µM) was added to some samples, and the release incubation was terminated 2 min later by placing the samples on ice.

Basal ACh release was significantly inhibited by the muscarinic agonists oxotremorine and carbachol in normal KR but not in calcium-free KR. Atropine had no effect per se on release under these conditions (less than 0.5 mg protein/ml), but blocked the actions of oxotremorine and carbachol. These receptor agonists also attenuated ACh release in the presence of K^+-depolarization or A23187 in concentration-dependent, atropine-sensitive manners. However, the kinetics of ACh release-modulation (± oxotremorine) observed for increasing levels of depolarization or A23187 differ in a manner that is consistent with the hypothesis that this muscarinic agonist is acting on voltage-dependent calcium uptake but not at some site distal to calcium entry. Further, oxotremorine attenuated ouabain-induced ACh release in normal KR in an atropine sensitive manner, but had no effect on the glycoside-elicited release observed in calcium-free KR. This result also suggests that the muscarinic modulation of ACh release depends on uptake through voltage-dependent calcium channels.

We also developed a liposomal delivery system to introduce large (e.g., acetylcholinesterase) or charged (calcium ions) compounds into synaptosomes, and then determine their effects on ACh levels, release, and release-modulation. Preliminary results indicate that liposomal introduction of calcium ions into nerve terminals releases ACh, and that oxotremorine reduces this release in the same manner that it inhibits A23187-induced release. Cholinesterase introduced into terminals in this manner reduces ACh levels and release, and experiments are in progress to determine the effects of this treatment on muscarinic release modulation.

283.11 UTILIZATION OF CHOLINE TRANSPORTED BY SODIUM-DEPENDENT, HIGH-AFFINITY CHOLINE CARRIERS FOR ACETYLCHOLINE SYNTHESIS: COMPARISON OF RAT AND GUINEA-PIG FOREBRAIN SYNAPTOSOMES. R. J. Rylett, T. J. Carlton* and E. H. Colhoun*. Department of Pharmacology, University of Western Ontario, London, Ont., Canada, N6A 5C1.

Controversy exists over the role of choline transported into synaptosomes by sodium-dependent, high-affinity carriers in the synthesis of acetylcholine (ACh). Some of the observed differences could be due to species variability in the parameters measured and the mechanisms involved. Largely, studies have involved the use of rat and guinea-pig brain, species which are known to differ with respect to the molecular forms (pI) of choline acetyltransferase. In the present report, we compare the kinetics of choline transport and the conversion of ^{3}H-choline to ^{3}H-ACh in resting and K^+-depolarized synaptosomes prepared from rat and guinea-pig forebrain. Analysis of choline transport over the ^{3}H-choline concentration range 0.1 to 100 μM revealed typical biphasic kinetics with apparent Michaelis constants, K_t, of 2 and 109 μM and V_{max} of 84.3 and 304.6 pmol/mg protein/4 min for rat forebrain synaptosomes; kinetics of transport for choline into synaptosomes prepared from guinea-pig brain did not differ significantly. Following incubation of anticholinesterase-treated synaptosomes with 1 μM choline, conversion of ^{3}H-choline to ^{3}H-ACh was quantitated by preparative HPLC separation of the choline metabolites and liquid scintillation spectrometry. Velocity for choline uptake in rat brain synaptosomes (^{3}H-choline=1 μM) was 20.2 pmol/mg protein/4 min of which 84% was transported by sodium-dependent processes; in comparison, choline transport velocity into guinea-pig synaptosomes was 18.7 pmol/mg protein/4 min, 79% of which was abolished in sodium-free (lithium substituted) medium. In regular K^+ (5 mM) medium, it was observed that 72% of ^{3}H-choline transported into rat brain synaptosomes by sodium-dependent processes was acetylated, while in guinea-pig only 57% of such choline was metabolized to ACh ($P<0.02$). Following K^+ (40 mM)-depolarization, 74.5% of ^{3}H-choline transported by sodium-dependent mechanisms in rat synaptosomes was acetylated, however, in guinea-pig synaptosomes this parameter was significantly increased to 70.3%. The net increase in ^{3}H-choline uptake following K^+-depolarization was 143.5% and 131% in rat and guinea-pig, respectively, relative to resting transport. Thus, in guinea-pig synaptosomes the percentage of choline transported via sodium-dependent carriers diverted to ACh synthesis is increased by depolarization of the nerve terminal. These results suggest that there may be differences underlying the coupling of choline transport to the enzymatic acetylation reaction and the utilization of exogenous choline in the synthesis of ACh in synaptosomes from brain of rat and guinea-pig.

(Supported by the Medical Research Council of Canada).

283.12 EFFECTS OF DFP ON THE RELEASE OF ACETYLCHOLINE: ROLE OF A PRESYNAPTIC MUSCARINIC RECEPTOR. T.G. Mattio*, E. Giacobini, J.S. Richardson*, (SPON: C. Su), Dept. Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62708

The albino rat iris contains a dense plexus of cholinergic nerve terminals whose cell bodies are located in the ciliary ganglion. This structure is a good model for the study of cholinergic function due to its homogeneity. Following characterization of the high affinity choline (Ch) uptake system, the electrically stimulated release of acetylcholine (ACh) was studied. ACh pools were labelled by uptake of ^{3}H-Ch for 10 min (1 uM). The irises were then rinsed and put in a release chamber modified from Potashner (1978). After a 10 min wash the tissue was stimulated by a 50 Hz, 20 mA, 5 ms square wave for 1 min while being superfused by oxygenated Elliots B buffer. The perfusate was collected into scintillation vials, after which 2 ml of cocktail was added and the radioactivity released was determined by liquid scintillation counting. The tritium released was expressed as a percentage of the total tritium present in the tissue at the time of release. We demonstrated that the tritium released was 95-100% ^{3}H-ACh. The release of ACh was found to be Na^+, Ca^{++} and temperature dependent. The addition of scopalamine (10^{-4}-10^{-5}M) increased the release of ACh up to 190% while, the addition of choline (10^{-3}M) decreased the release of ACh. This decrease in release was reversed by the addition of 10^{-6}M scopolamine, demonstrating the presence of a presynaptic muscarinic receptor, as has been described in other tissues. The addition of the irreversible cholinesterase inhibitor diisopropyl fluorophosphate (DFP) (10^{-4}, 10^{-5}, 10^{-6}M) into the superfusion buffer resulted in a significant decrease in the stimulated release of ACh with esterase activity inhibited by more than 90%. DFP at 10^{-7} and 10^{-8}M inhibited esterase activity by 60 and 40%, respectively, but had no effect on the release of ACh. This decrease on the release of ACh was found to be totally reversible with the addition of 10^{-6}M scopolamine into the buffer. The decrease in release of ACh by DFP can be attributed to the accumulation of ACh in the synaptic cleft and consequently its agonistic effect on the presynaptic muscarinic receptor therfore decreasing release of ACh. The muscarinic antagonist scopolamine, at a concentration where itself does not affect release, was able to totally reverse this effect. This data demonstrates the presence of a presynaptic muscarinic receptor on cholinergic terminals in the rat iris and suggests a mechanism by which DFP decreases the release of ACh from cholinergic terminals. (Supported by Air Force grant #81-0229 and 83-0051.)

283.13 SECRETION OF ^{3}H-ACETYLCHOLINE FROM GUINEA-PIG ILEUM MYENTERIC PLEXUS IS ENHANCED BY INHIBITORS OF PHOSPHODIESTERASE. P. Alberts* and Å. Sellström*. (SPON: P. Greengard). Division of Experimental Medicine, National Defense Research Institute, S-901 82 Umeå, Sweden.

The secretion of acetylcholine (ACh) is regulated by presynaptic muscarinic feedback inhibition. The possible involvement of endogenous cyclic nucleotides in this control was investigated using two inhibitors of phosphodiesterase. The ACh stores of the cholinergic nerves of the myenteric plexus of the guinea-pig ileum longitudinal muscle preparation were labelled with ^{3}H-choline. The preparation was mounted in an organ chamber and superfused with Tyrode solution containing hemicholinium-3 (10^{-5} M) and eserine (10^{-5} M). Stimulation was with trains of 150 shocks (0.5 ms, 120 V) at a low frequency (0.5 Hz). The results are expressed as the evoked fractional secretion of total ^{3}H. Addition of 3-isobutyl-1-methylxanthine (IBMX, 2.25 mM) enhanced the evoked secretion of ^{3}H-ACh by 99 ± 28 % (n=6, $p<0.001$). From the effects of IBMX (1-5 mM, n=7) the concentration yielding half-maximal enhancement (K_m) was determined to be 2.0 mM. The maximal increase over the control level at infinitely high concentration of IBMX (V_{max}) was estimated to be 190 %. Furthermore, the effects of IBMX (1 or 2 mM) were not altered by atropine (10^{-5} M). A structurally different inhibitor of phosphodiesterase, SQ 20,006, also slightly enhanced the ^{3}H-ACh secretion but within a very narrow concentration range. The secretion was enhanced by 40-110% by SQ 20,006 (0.3-0.5 mM). Above this range the secretion was enhanced drastically, about 10-fold, and was probably not related to the inhibition of phosphodiesterase. The results suggest that endogenous cyclic nucleotides are not involved in muscarinic "autoinhibition" of ^{3}H-ACh secretion in guinea-pig ileum myenteric plexus. However, it is conceivable that adenosine 3',5'-cyclic monophosphate may be involved in the enhancement of evoked ^{3}H-ACh secretion caused by activation of other receptors.

283.14 ACTIVATION OF ACETYLCHOLINE SYNTHESIS IN THE ABSENCE OF RELEASE: DEPENDENCE ON SODIUM, CALCIUM AND THE SODIUM PUMP. R.I. Birks. Department of Physiology, McGill University, Montreal, Canada H3G 1Y6.

Following a 15 min inhibition of the sodium pump in the cat superior cervical ganglion by perfusion with K-free Locke solution, a 10 min recovery in normal Locke produced a 51% increase in acetylcholine stores. The increase in stores occurred without increase in acetylcholine release. Thus this procedure of pump inhibition followed by recovery selectively activates acetylcholine synthesis. The increase in stores, which occurred entirely during the 10 min recovery period in which the sodium pump was reactivated, represents a rate of synthesis of acetylcholine of 5.1% of stores per min; equal to the maximum rate that can be achieved during high frequency preganglionic nerve stimulation. The increase was not affected by substituting isethionate for chloride in the perfusion fluids. It was prevented by reducing sodium to 25 mM in the K-free Locke and also prevented by omitting calcium from the perfusion fluids. It is concluded that the selective activation of acetylcholine synthesis following the pause in sodium pumping was a direct result of an increased sodium pump rate and an increase in internal calcium in the nerve terminals. It is proposed that similar ionic events produced by repetitive nerve impulses likewise activate acetylcholine synthesis independently of release of transmitter or depletion of stores.

283.15 THE ANTICONVULSANT ACTIVITY OF HEMICHOLINIUM-3, A HIGH AFFINITY CHOLINE UPTAKE INHIBITOR. J.A. Miller* and J.A. Richter. Departments of Pharmacology and Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

Barbiturates and benzodiazepines inhibit high affinity choline uptake (HACU) in doses that correlate with their anticonvulsant potency. We have hypothesized that this inhibition is responsible for their anticonvulsant activity (Miller, J.A. and Richter, J.A., Neuroscience Abs. 8: 649, 1982). To test this hypothesis we examined an inhibitor of HACU for anticonvulsant activity. Hemicholinium-3 (HC-3) given intracerebroventricularly (i.c.v.) to mice one hour prior to intraperitoneal (i.p.) administration of picrotoxin, protected against convulsions.

Under light ether anesthesia, HC-3 was given to male ICR-Swiss mice i.c.v. by freehand injection (Haley, T.J. and McCormick, W.G., Br. J. Phar. 12: 12, 1957) into both lateral ventricles. A total of 3 µg of HC-3 (1.5 µg per ventricle) was given to each animal in a total volume of 10 µl (5 µl per ventricle). Previous tests showed that under these conditions this amount of HC-3 was nearly the maximal nonlethal dose. Control animals received 5 µl of saline in each ventricle. One hour after pretreatment, picrotoxin (8 mg/kg) was administered i.p. A significant ($p < .02$) protective effect of HC-3 was observed. Of the saline pretreated animals 93% convulsed while only 53% of the HC-3 pretreated animals convulsed. Mice identically pretreated with either HC-3 or saline were sacrificed one hour postinjection so acetylcholine (ACh) levels could be measured in the cortex and hippocampus. ACh was extracted in a formic acid/acetone mixture and assayed by a radioenzymatic method using choline kinase and γ-^{32}P-ATP. A significant ($p < .05$) 75% decrease in ACh levels was observed in the HC-3 treated mice relative to saline controls. This indicates that the compound was effective in inhibiting HACU which is necessary for ACh synthesis. We plan to examine further the anticonvulsant effect of HC-3 in rats. Other future experiments will focus on the major ascending cholinergic pathways eminating from the medial septum and nucleus basalis. We expect lesions of these pathways to afford anticonvulsant activity. (Supported by PHS Grant DA-00796).

283.16 EFFECTS OF ORAL CDP-CHOLINE ADMINISTRATION ON PLASMA AND BRAIN CHOLINE LEVELS IN THE RAT. P.G. Holbrook*, I. Lopez G-C* and R.J. Wurtman (SPON E. Spindel). Laboratory of Neuroendocrine Regulation, Massachusetts Institute of Technology, Cambridge, MA 02139

Administration of cytidyl diphosphocholine (CDP-choline), an intermediate in the pathway which incorporates free choline into phosphatidylcholine (PC), reportedly activates brain tyrosine hydroxylase (Martinet M., Fonlupt P., Pacheco H., Biochem. Pharmacol., 30: 539-541, 1981) and has other neurochemical effects in rats. We examined the effects of oral CDP-choline administration on plasma and brain choline levels in the rat. In a pilot study we compared the effects of CDP-choline (2.25 g/kg) with equimolar choline chloride or PC on plasma choline levels in rats with jugular cannulas. CDP-choline elevated plasma choline levels by 50% after 4 hours; its time course being more like that of PC than choline chloride. In another experiment, we measured whole-brain choline levels in rats given CDP-choline (1.5 g/kg) or equimolar choline chloride and killed after 1, 5 and 24 hours by focussed microwave irradiation to the head. Whole-brain choline was elevated relative to controls at all times tested in both choline treated and CDP-choline treated animals. Peak values occured at 5 hours; levels returned towards baseline by 24 hours. Since administration of a choline dose that raises brain choline can also increase acetylcholine levels (Cohen E.L., Wurtman R.J., Life Sci. 16: 1095-1102, 1975) and thereby activates caudate tyrosine hydroxylase (Ulus I., Wurtman R.J., Science, 194: 1060-1061, 1976) our data suggest that at least some of CDP-choline's neurochemical effects result from its providing a source of plasma choline.

BRAIN METABOLISM I

284.1 EVALUATION OF A VIDEO-BASED IMAGE PROCESSING SYSTEM FOR QUANTITATIVE AUTORADIOGRAPHIC DENSITOMETRY. J.H. Kulick*, P. Ramm* and B.J. Frost (Spon. R. D. Andrew).

We have evaluated the densitometric accuracy of a video-based image processing system, used for quantitative densitometry. The video camera (VC), a Hammamatsu C-1000 fitted with an E5001 chalnicon was interfaced to a Grinnell GMR 270 Display system (512 x 512 visible resolution). All readings were made using the PANDA (Program for Analysis of Digitized Autoradiographs) routines, developed in this laboratory. The VC scanned a Kodak photographic step tablet, consisting of 0.10 D steps (+- 0.01 D) from 0.05 - 1.28 D (read in % transmission, 89.0 - 5.2%), The steps were selected to span the density range found in most autoradiographs. Shading error and densitometric nonlinearity (gamma) were software-corrected. Averaging of 256 frames/image provided 8 bit densitometric resolution. Between scans, the entire system was turned off, turned on again, and recalibrated. This procedure was repeated 5 times with the VC and, without application of shading and linearity corrections, with an Optronics P-1700 scanning microdensitometer (SMD).

Regression of the % transmission values obtained with the VC, upon actual transmission values yields the equation $Y = -0.719 + 1.01X$. Correlation between the paired values is essentially perfect ($r = 0.999$). Standard deviations across sessions did not exceed 1% transmission. These data are very comparable to those obtained by repeatedly scanning the wedge with the SMD. Thus, this video-based image processing system exhibits near-perfect densitometric accuracy and linearity, and excellent between session repeatability.

Quantitative densitometry is usually performed with a microscope-mounted photometer or SMD. Although VC advantages include low cost, high speed, application flexibility and continuously variable optical magnification, its densitometric nonlinerities and lack of dynamic range limit its usefulness for quantitative densitometry. The present data suggest that a high-quality VC can be an appropriate scanning device for autoradiographic quantitative densitometry under the following conditions: 1) software corrections for shading and gamma are applied; 2) the autoradiographs are exposed so as to yield images with appropriate dynamic range; 3) a procedure (such as frame averaging) is used for improving signal-to-noise ratio; 4) data are sampled at magnification such that the modulation transfer function approaches unity.

284.2 A DENSITOMETRIC STUDY OF FROG LINGUAL NERVE COMPONENTS AS REVEALED BY 2DG AUTORADIOGRAPHY. Howard J. Bryant and Francis A. Kutyna, Department of Physiology, Uniformed Services University, Bethesda, MD 20814.

Neuronal components of the gustatory division of the glossopharyngeal (IX) nerve in the frog were activated through electrical stimulation of the lingual branch. Antidromic action potentials were initated in the axons of the motor constituents of the nerve and invaded the cell bodies located in the brainstem and first sympathetic ganglion. Orthodromically activated sensory afferents resulted in increased metabolism in the gustatory component. Utilizing the 2-deoxy-D-^{14}C glucose (2DG) method for functional activity in the nervous system, autoradiographs of brain sections and ganglia were analyzed using a beam splitting optical microdensitometer. Optical densities of active brain areas were normalized to a stable brain area (the preoptic nucleus) which allowed comparisons of the relative activities of the various nerve components.

The microdensitometer used to measure autoradiograph optical densities was constructed by modifying a standard laboratory compound microscope equipped for photography. A photo-transistor detector was mounted in an adapter in place of the existing camera. The microscope magnification and the position of the detector were adjusted so that the microdensitometer was sensitive to an area 1.5 mm in diameter on the film. The autoradiograph was positioned under the sensitive area of the microdensitometer by viewing through the eyepieces of the microscope or by observing the projected image of the film on a ground glass screen that replaced one of the eyepieces. Modifications to the microscope converting it into a microdensitometer do not permanently preclude its use as a standard optical microscope.

The output of the detector was amplified and displayed on a digital meter by a simple electronic circuit. By use of a calibrated optical density step tablet, the meter readings can be converted into optical densities. Alternatively, the meter readings may be converted into tissue slice 2DG concentrations by means of 2DG standards exposed on the autoradiograph film.

Unilateral electrical stimulation (20 Hz, 1 ms, 4V) over a period of 45 min produced measurable increases in 2DG uptake ratios of (1) the ipsilateral solitary nucleus, (2) the ipsilateral IX motor nucleus, (3) the pneumogastric ganglion, and (4) the first sympathetic ganglion. Functionally, these structures correspond to (1) the primary sensory relay nucleus for the gustatory afferents, (2) the visceral efferent component to the tongue, (3) the cell bodies of the primary gustatory afferents, and (4) the postganglionic cell bodies of the sympathetic efferents to the tongue. Supported in part by USUHS Grant C07606.

284.3 KINETICS OF CEREBRAL GLUCOSE UPTAKE AND BLOOD FLOW USING THE SINGLE PASS TECHNIQUE IN UNANESTHESIZED RATS. Leon Braun*, Leonard Miller*, William Pardridge and William Oldendorf, Research Service, Brentwood Hospital, Veterans Administration and Depts of Medicine and Neurology, UCLA School of Medicine, Los Angeles, Calif. 90073.

Previous investigations of the kinetics of glucose transport across the blood-brain barrier (BBB) using the single pass technique described by Oldendorf (Res Methods in Neurochem 5,1981) made use of anesthetized rats because of the ease of surgical manipulations. However, the anesthesia itself alters blood flow with subsequent effects on the rate of glucose transport across the capillary endothelia. Thus proper assessment of accurate kinetic parameters is difficult under these circumstances. The present investigation reports on the cerebral uptake of glucose and blood flow in four different brain regions using unanesthetized rats. Rats were anesthesized with pentobarbital (50mg/kg), the external carotid exposed and a 12cm length of PE-10 tubing filled with heparin inserted and tied into the external carotid. The cannula was externalized through the back of the neck and animals were allowed to recover overnight in separate chambers. On the following day the animals were injected through the cannula with a 200ul bolus of Hepes- buffered Ringers solution ,pH7.5, containing (C^{14}) glucose and (H^3) water reference. For blood flow determinations the washout of C^{14} butanol was followed for up to two minutes. Our results were:

Region	BUI (%)	E_{water} (%)	F (ml/min/g)	V_{max} (umol/min/g)	K_M (mM)	K_D (ml/min/g)
Hippocampus	26	59	1.16	1.07	6.3	0.032
Caudate	25	56	1.55	1.95	8.6	0.020
Cortex	26	50	1.65	1.43	6.8	0.030
Thalamus	28	60	1.37	1.55	7.1	0.052

(BUI=Brain Uptake Index, E=Extraction, F=Flow) Each value is the mean of at least 3 separate observations with an average variation of approximately 33%. From the data we were able to calculate a mean glucose extraction of 15%. The K_M and K_D were not significantly different from those obtained using barbiturate anesthetized rats (Pardridge et.al., J.Neurochem.38,1982). Cerebral blood flow and V_{max},however, showed significant differences and were 2 to 3 times higher in the present study. The present technique should prove valuable in further investigations using unanesthetized animals.

284.4 LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU) IN RATS IS UNAFFECTED BY IMMOBILIZATION STRESS OF EXPERIMENTAL PREPARATION. T.T. Soncrant*, H. Holloway,* S. Carlson,* S.I. Rapoport (SPON: G.P. Redmond). Laboratory of Neurosciences, National Institute on Aging, NIH, Balto. City Hospitals, Baltimore, Maryland 21224.

In the awake rat immobilization stress is accompanied by an increase in circulating catecholamines (CA) and by alterations in brain neurotransmitter turnover (Thierry et al, Nature 263:242, 1976), though regional cerebral blood flow (rCBF) remains essentially unchanged (Ohata et al, J Cereb Blood Flow & Metab 2:373, 1982). Recently, however, Bryan et al (Am J Physiol 244:C270, 1983) reported large elevations in LCGU in paralyzed, ventilated rats when compared to chronically catheterized, awake animals. In the "standard" rat preparation for LCGU determination with the 14-C-deoxyglucose (DG) method of Sokoloff et al (J Neurochem 28:897, 1977), vascular catheters are placed surgically, and hindlimbs are restrained for 2-6 h before measurement of LCGU. Recent surgery as well as limb restraint, however, can produce moderate increases in plasma CA (Popper et al, J Pharm Exp Ther 202:144, 1977).

We examined the contribution of these relatively modest stresses, routinely employed in the DG procedure in rats, to basal LCGU in awake 3 mo Fischer rats. Control rats were catheterized 24 h before DG was injected, and were unrestrained. A second group was catheterized 24 h before DG, and hindlimbs were restrained 4 hr before DG. The third group was the "standard" preparation in which catheters were placed and rats were immediately immobilized 4 h before DG.

No significant difference in LCGU among the three groups was seen in any of the 27 representative regions studied. Our LCGU values are consistent with literature DG determinations in young, awake rats. Moreover, they agree, except in auditory regions, with rates of LCGU reported by Bryan et al using the 14-C-glucose method in rats catheterized and isolated 1 wk prior to study.

These results demonstrate that neither hindlimb restraint nor same-day surgery affects determination of LCGU using the DG technique. Findings are consistent with studies of rCBF during immobilization. Prolonged recovery from catheter implantation and an unrestrained animal preparation appear to be unnecessary for determination of basal LCGU in awake rats.

284.5 COMPARISON OF 5-THIOGLUCOSE AND GOLD THIOGLUCOSE ACTION ON INSULIN HYPOGLYCEMIC CONVULSIONS AND CYTOTOXIC LESION FORMATION Mary Ann Marrazzi, James Brown*, Grace Yabut* and Joyce Wright*. Dept. Pharmacology, Wayne State Univ. Sch. Medicine, Detroit, MI 48201

Gold thioglucose (GTG), injected intraperitoneally in female CBA/J mice, causes a cytotoxic lesion focused in the ventromedial-arcuate hypothalamus (VMH). Like other VMH lesions, it causes hyperphagia and obesity. We have previously shown that in addition it changes the sensitivity to insulin hypoglycemic convulsions in a biphasic manner [Luby et al., J. Pharmacol. Exper. Therap. 219: 258, 1981]. The sensitivity (% convulsions in a group) is decreased at early times (16-24 hours) but increased at later times (1-2 weeks) after the single GTG dose. For both effects, the difference must be in the brain's convulsive response to equal hypoglycemia, rather than in the degree of hypoglycemia in response to insulin, since the blood glucose levels are the same in all groups. Metrazol induced convulsions are not affected at either time, indicating that the effects are not non-specific ones on the generalized convulsive threshold or on the response to stress. Both effects are prior to the eventual gain in body weight. The histological damage in the VMH is clearly visible with cresyl violet by the time the first functional change in the sensitivity to insulin hypoglycemic convulsions could be detected. These results suggest a relatively discrete brain region involved in adjusting brain function in response to insulin hypoglycemia. Such a regulatory center could account for the lack of generalized power failure, in previous reports, indicated by the lack of depletion of biochemical energy reserves despite convulsion and coma.

5-Thioglucose (5-TG) substitutes for GTG in decreasing the sensitivity to insulin hypoglycemic convulsions at 24 hours, but has no effect at 1 week. Like with GTG, blood glucose measurements indicate that the difference is in the brain's convulsive response to equal hypoglycemic challenge. Like with GTG, Metrazol induced convulsions are not affected. However unlike GTG, no lesion is visible in the VMH with cresyl violet and no long term obesity results. Thus 5-TG produces the early decrease in sensitivity to insulin hypoglycemic convulsions without the VMH lesion. Other brain regions were examined for histological damage visible with cresyl violet for overlap of a 5-TG lesion and a secondary site of GTG lesions. No 5-TG lesion was found. The sulfur substitution of 5-TG is in the pyranose ring rather than the β-D-position as in GTG. However, 5-TG has glucoregulatory effects - producing short term hyperglycemia and hyperphagia, mediated by some area around the fourth ventricle [Ritter et al., Science 213:453, 1981]. The area postrema was of particular interest because it could be the site of this 5-TG glucoregulation and has been reported to show minor histological damage with GTG. However, it also did not show a 5-TG lesion. (NIH#RR 08167& 5R01 AM 21476).

284.6 BRAIN EXTRACELLULAR POTASSIUM AND ENERGY METABOLISM IN SHORT-TERM ISCHEMIA. T. Yamaguchi*, Y. Yasumoto*, F. A. Plowman*, W. D. Lust, H. G. Wagner and I. Klatzo. Lab. Neuropath. and Neuroanat. Sciences, Lab. Neurochem., Biomed. Eng. and Instru. Branch and Lab. Neurophysiol., NINCDS, NIH, Bethesda, MD 20205

We report here on the findings of a study on the relationship between extracellular potassium concentration ($[K^+]e$) and the levels of high-energy phosphates (ATP plus P-creatine) in gerbil brains both during and after 5 min of bilateral ischemia. The gerbils were anesthetized with 40 mg/kg pentobarbital (ip) and both common carotid arteries were occluded for 5 min. The field potential (Fp) and $[K^+]e$ were monitored using ion-sensitive electrodes which were inserted either into the cortex or hippocampus. At various times during both ischemia and recirculation, the electrodes were withdrawn and the brains were frozen in situ with liquid nitrogen. The P-creatine and ATP were measured in the CA 1 and CA 3 regions of the hippocampus as well as in the cerebral cortex using the luciferin-luciferase technique.

The $[K^+]e$ changes during ischemia and recirculation occurred in essentially 5 stages: 1) a 2-fold increase in the first 100 sec of ischemia, 2) followed by a rapid increase to 33.8 ± 3.3 mM in a matter of seconds, 3) a gradual increase to a maximum of 67.5 ± 4.6 mM at the end of ischemia, 4) a 25 mM drop in $[K^+]e$ in the first 4 min of recirculation and 5) full restoration of $[K^+]e$ to control values (3.5 ± 0.2 mM) by 8 min of recirculation. The anoxic depolarization was evident in stage 2 and repolarization in stage 5. In stage 1, the high-energy phosphates decreased by more than 50% and thereafter dropped to less than 30% of control at the end of the ischemic episode. Based on previous results, a greater fall would have been expected; however, this may be attributed to the use of an anesthetic which is known to depress the metabolic rate of the brain. The high-energy phosphates recovered to approximately 70% of control values in stage 4 and were completely restored by the end of stage 5. While the results shown here are taken from the hippocampus, the changes in the cerebral cortex were similar, although there were subtle differences in both the time course and magnitude of the response.

The fact that a portion of the high-energy phosphate pool is lost prior to the anoxic depolarization and conversely that the extracellular potassium is reaccumulated when the energy status of the tissue is somewhat depressed could be explained by the existence of a critical concentration of ATP necessary for the maintenance of potassium homeostasis.

284.7 2-DEOXYGLUCOSE STUDIES OF CEREBRAL ISCHEMIA. Z. Ye*, Y.C. He* and M.C. Yu*(SPON: H. Lowndes). Dept. of Anatomy, New Jersey Medical School, Newark, N.J. 07103

Glucose utilization in the cerebrum was studied after ligation of the right common carotid artery for 1 to 3 hrs in gerbils weighing about 60 g. A total of 60 animals was subjected to carotid ligation of which 15 developed neurological signs of brain injury (turning in circles, splaying or seizure). The onset of neurological symptoms varied considerably among individual animals, but could be correlated with the absence of collateral circulation by latex tracing of the cerebral vessels, and by the reduction of glucose utilization in the lesioned hemisphere. Those gerbils which failed to develop symptoms were found to have collateral circulation and no difference in glucose utilization between the lesioned and intact hemispheres. The animals were killed at 3 hrs, 24 hrs and 4 days postligation. Each animal was injected intraperitoneally with 2-deoxy-D-glucose (1uC/10g body weight) 45 minutes prior to sacrifice. Serial coronal sections were exposed to X-ray films for 7 days and the autoradiograms were read with a digital densitometer with a 0.1 mm aperture. The following brain regions were examined: frontal, parietal, occipital cortices, dorsal hippocampus and caudate nucleus. Glucose utilization in various brain regions was inferred from the optical density (O.D.) on the X-ray films, and the O.D. of the lesioned hemisphere was expressed as a percentage of that of the intact (control) hemisphere. At 3 hrs postligation, glucose utilization on the ischemic hemisphere was reduced to less than 50% of the control, with the frontal cortex and caudate nucleus showing the greatest reduction. At 24 hrs postligation, glucose utilization showed a small increase in all brain regions examined, and by day 4, it had returned to about 90% of control values in the parietal and occipital cortices and the hippocampus. The glucose utilization of the frontal cortex and caudate nucleus however, still lagged much behind. These data indicate considerable variation among different brain regions to ischemic insult and also the rate of recovery. These metabolic changes are being correlated with morphological investigations.

(Supported by NIH Grant 1 R01HD12089 to M. Yu)

284.8 Differential Sensitivity of Pyramidal and Granule Cell Neurons to Anoxic Damage in Hippocampi from Young Rats. Ira S. Kass and Peter Lipton. Dept. of Physiology, Univ. of Wisconsin, Madison WI 53706.

Very young animals are less susceptible to neuronal damage during cerebral anoxia. We used the hippocampal slice preparation as a model to investigate this phenomenon. Previous experiments on young animals indicated that the fall in ATP levels (measured in whole slices) during 10 minutes of anoxia did not correlate with the recovery of the orthodromically evoked population spike from the dentate granule cells. Thus in spite of a large fall in ATP the spike from young animals recovered. This result was in sharp contrast to the adult; where reduced ATP levels during anoxia correlated with irreversible damage. These results suggested that either the young tissue was protected against irreversible signal loss in spite of a profound drop in ATP or ATP levels measured in the whole slice did not mirror the levels found in the dentate region during anoxia.

In contrast to the dentate region, the evoked response from the CA1 region in young animals did not recover from 10 minutes of anoxia; thus, there is differential susceptibility of the CA1 pyramidal cells and the dentate granule cells in the young animals. We next microdissected lyophilized hippocampal slices and determined that there was a more profound drop in ATP levels in the CA1 region than in the dentate region during anoxia. The ATP levels from the CA1 region dropped to 1.3 ± .1 nM/mg dry wt. (± S.E.M.) while the dentate region dropped to 1.9 ± .2. Thus the ability to recover correlated with ATP levels during anoxia. When ATP levels in the CA1 region during anoxia were elevated from 1.3 ± 1 to 2.1 ± 1 by preincubating the slices for 4 hours in 25 mM creatine the population spike from the CA1 region recovered from 10 minutes of anoxia. Untreated slices showed no recovery from anoxia while the creatine treated slices showed 48 ± 9% recovery. Thus, reducing the fall in ATP during anoxia diminishes the irreversible anoxic damage.

In order to see if the higher anoxic levels of ATP in the dentate region could account for its ability to recover we examined the effects of 15 minutes of anoxia. This reduced ATP levels in the dentate region during anoxia to 1.4 ± .1 nM/mg dry wt. Coincident with this reduced ATP there was a greatly reduced ability of the dentate region to recover from the anoxia. Thus, as for CA1, when ATP was reduced to a certain level the dentate region did not recover.

Thus in the young hippocampus, cell populations have different susceptibilities to anoxia. This susceptibility correlates with the developmental stage of the region. It appears that the differential susceptibility can be explained by the different levels to which ATP falls in the different regions during anoxia.

284.9 Effects of an Adenosine Analogue on Mortality following Permanent Unilateral Carotid Ligation in the Gerbil. F. W. Marcoux, J. J. Cordon* and J. M. Weaver*. Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

The Mongolian gerbil is now well documented as a useful animal model for abrupt, occlusive stroke. Thirty to sixty percent of gerbils are reported to die after unilateral carotid occlusion in the unanesthetized or lightly anesthetized state. In our hands, 50% of gerbils die within 48 hours after ligation during light ketamine anesthesia. The variable mortality rate is presumed to be the result of variable collateral circulation between animals; this is true of other animal stroke models, and probably stroke in humans as well.

We have evaluated the adenosine analogue, N^6-cyclohexyladenosine (CHA), for its effects on stroke mortality in the gerbil model, when given after the stroke. Gerbils lightly anesthetized with ketamine (125 mg/kg, IM) underwent permanent carotid ligation with body temperature maintained at 37-39°C. Drug treatment began at 30 minutes after carotid ligation and was continued during the 48 hour post-ligation observation period. A reduction in mortality rate in the drug treated compared to control gerbils was interpreted as protection against stroke.

In preliminary trials, CHA was administered 30 minutes after carotid ligation in dose levels of 0.1, 1.0 and 10.0 mg/kg IP and then equivalent doses were given subcutaneously 3 times a day for the next 2 days. Mortality rate was established for each group at the end of the 2 day treatment period.

Gerbils given 0.10 mg/kg CHA revealed a mortality of 3/10 during the 48 hour post-ligation period. 19/38 (50%) of untreated gerbils died within this period (2 died after 2 days post-ligation). This dose of CHA mildly sedated gerbils with carotid ligation but had little or no behavioral effect on sham operated, non-stroke gerbils. 1.0 mg/kg CHA treatment revealed a mortality of 3/10 during the 48 hour post-ligation period; 1 died on day 3. This dose of CHA produced moderate sedation in stroke and non-stroke gerbils. Gerbils treated with 10.0 mg/kg CHA revealed a mortality of 2/10 in the first 48 hours post-ligation; however, the other 8 animals died on day 3. This dose of CHA severely sedated stroke and non-stroke gerbils.

These data suggest that this adenosine analogue reduces mortality following unilateral carotid ligation in gerbils; the potential contribution of hypothermia to this effect is under investigation in larger trials.

284.10 REGIONAL METABOLITES: PREDICTOR OF REGIONAL INJURY IN TRANSIENT ISCHEMIA. R. Busto*, M.D. Ginsberg, E. Martinez*, I. Valdes*, O.F. Alonso. Cerebral Vascular Disease Research Center, Dept. of Neurology, University of Miami School of Medicine, Miami, FL 33101.

To determine whether postischemic metabolic changes might reflect regional neuronal damage, rats were exposed to 30 min of global forebrain ischemia by bilateral carotid and vertebral artery occlusions. Brain levels of adenylates, phosphocreatine, glucose, lactate, and pyruvate were assessed in 3-5 mg samples from multiple regions at 30 min, 1 hr, and 4 hr of normotensive postischemic recirculation. Control brains showed no regional differences in metabolites. Regional heterogeneities of ATP were seen during ischemia but not during recirculation. During ischemia, ATP was lowest (4-5% of control) in neocortical regions, but 14-20% of control in hippocampus and striatum. Despite return to 70-80% of control by 30 min of recovery, ATP remained slightly depressed even after 4 hr of recirculation. Supranormal PCr values were seen at 30 min and 1 hr of recirculation in all regions except hippocampus. Lactate rose 10-12 fold during ischemia, declined to normal by 1 hr of recirculation, but secondarily increased significantly (1.5-2.3 X control) at 4 hr of recirculation in hippocampus and striatum. Thus, hippocampus and striatum, the sites of preferential neuronal damage in this model, show 1) higher ATP during ischemia, and 2) secondary lactic acidosis during recirculation. The decline of ATP observed at the end of ischemia does not of itself predict the ultimate degree of derangement of energy metabolism. Rather, postischemic factors appear have an important role in determining the development of tissue damage.

(Supported by USPHS Grant 05820-17.)

284.11 IN VIVO ^{31}P AND HIGH RESOLUTION ^{1}H NMR STUDY OF EXPERIMENTAL HYPOGLYCEMIC ENCEPHALOPHY, K.L. Behar, O.A.C. Petroff, J.A. den Hollander, M. Stromski, J.W. Prichard and R.G. Shulman (SPON: J.S. Ebersole). Dept. Molecular Biophysics and Biochemistry and Dept. Neurology, Sch. of Med., Yale Univ., New Haven, CT 06511.

Insulin-induced hypoglycemia was studied by ^{31}P nuclear magnetic resonance in rabbit brain at 1.89 Tesla and by high resolution ^{1}H NMR in the rat brain at 8.3 Tesla using radio-frequency surface coils. Animals were given phentolamine and/or propranolol to block catechol mediated stress-induced hyperglycemia. Animals were tracheostomized, paralyzed, and mechanically ventilated with 30% O_2 in N_2O. Arterial pO_2, pCO_2, pH and blood pressure were recorded (in rabbits) while the EEG and EKG were monitored in both animals.

^{31}P NMR spectra recorded from the brains of rabbits revealed decreases of 20 to 50% in phosphocreatine levels with corresponding increases in P_i during electrocerebral silence (blood glucose $\leq$ 1mM). Intracellular pH (determined from the pH dependence of the chemical shift of P_i in the ^{31}P spectrum) was observed to rise during this interval by $\sim$ 0.2 pH units. The intracellular alkalosis appeared to be independent of the magnitude of change in phosphocreatine levels. This evidence--as well as the direction of change in pH_i--indicates that the fall in phosphocreatine during hypocapnic hypoglycemic coma was not due to the effect of H^+ on the equilibrium governed by the creatine kinase reaction. Upon recovery by intra-venous glucose administration, phosphocreatine recovered to near normal levels while P_i declined to a supra-normal steady state level. The intracellular alkalosis was reversed to an acidosis during recovery as blood glucose levels rose beyond $\sim$ 4mM.

The recent extension of in vivo high resolution ^{1}H NMR spectroscopy to brain studies in our laboratory has allowed us to access relative changes in certain organic acids in the rat brain during hypoglycemia. A resonance tentatively assigned to glutamate was seen to fall during the isoelectric period. Lactate levels during ischemia (cardiovascular collapse) were considerably lower than those seen during hypoxia or ischemia in the normoglycemic rat. The apparent lack of pH_i regulation during hypoglycemic coma is consistent with the observed depletion of glutamate and the reduced amounts of substrate for lactate production.

284.12 CEREBRAL BLOOD FLOW DECREASES DURING ACUTE HYPERGLYCEMIA IN THE RAT: EVIDENCE AGAINST AN OSMOTIC MECHANISM. R.B. Duckrow, D.C. Beard* and R.W. Brennan*. Division of Neurology, College of Medicine, The Pennsylvania State University, Hershey, PA 17033.

The presence of hyperglycemia prior to transient cerebral ischemia increases stroke related morbidity and mortality in experimental animals and perhaps in humans as well. However, little is known of the effect of hyperglycemia on regional cerebral blood flow (rCBF). Initial studies suggest that brain blood flow decreases during acute elevations of blood glucose content. We studied this in awake, partially restrained rats to determine if the acute increase in plasma osmolality which accompanies hyperglycemia could explain this flow decrease. Also, we studied the regional distribution of this phenomenon.

Fed adult male Sprague-Dawley rats were lightly anesthetized with halothane and nitrous oxide to allow placement of arterial, venous and intraperitoneal catheters through locally anesthetized incisions. The hips and hind legs were wrapped in a snug plaster cast, and anesthesia was discontinued. After recovery, either d-glucose, d-mannitol (both 50% solutions) or normal saline was injected intraperitoneally (7 ml/kg). Twenty minutes later rCBF was determined using ^{14}C-iodoantipyrine and quantitative autoradiography. Plasma glucose levels were determined using the glucose oxidase method and osmolality was measured by freezing point depression.

Six animals were assigned to each treatment group. Arterial blood pressure, P_aCO_2 and P_aO_2 were similar in each group at the time rCBF was determined. Plasma glucose levels were 11 ± 0.6 mM (SEM) after saline injection, 13 ± 0.3 mM after mannitol injection and 39 ± 4 mM after glucose injection. Normal saline did not alter plasma osmolality (control value: 287 ± 2 mOsm). However, both mannitol and glucose treated animals had plasma osmolalities of 307 ± 5 mOsm. In all brain regions studied rCBF was reduced during hyperglycemia. The mean flow reduction was 24% below control values. On an individual basis, 11 of 24 brain regions had significantly lower blood flow (t-test, $p<0.05$). However, there was no regional variation in the degree of blood flow reduction. Mannitol induced hyperosmolality did not alter rCBF from control values.

It is important to recognize the plasma glucose level as a factor which can influence cerebral blood flow in normal animals. This data suggests that this effect can be present during moderate elevations of plasma glucose and that it cannot be completely explained by elevations in plasma osmolality. If a similar influence is present during stroke, hyperglycemia-induced hypoperfusion may potentiate other mechanism which increase ischemic neuronal damage.

284.13 STIMULUS-EVOKED OXIDATIVE RESPONSES OF BRAIN CYTOCHROMES MEASURED IN SITU BY RAPID SCANNING SPECTROPHOTOMETRY. J.C. LaManna, T.J. Sick, S.M. Pikarsky* and M. Rosenthal. Case Western Reserve Univ and Univ of Miami Schools of Medicine, Cleveland, OH 44106 and Miami, FL 33101.

The reduction/oxidation (redox) status of the terminal member of the mitochondrial respiratory chain, cytochrome c oxidase (cyt a,a_3), in intact brain under normoxic conditions remains controversial. This controversy is based upon spectrophotometric findings that cyt a,a_3 is fully oxidized to very low oxygen tensions in isolated mitochondria but that this cytochrome is partially reduced in intact, normoxic brain, heart and kidney. These data have been questioned because of possible complications to dual wavelength reflection spectrophotometry of light absorption by hemoglobin. In the present study, reflectance spectra were recorded from cerebral surfaces of rats anesthetized with pentobarbital by means of a spectrograph and a detecting linear diode array. The array was scanned at 3 Hz to provide complete spectral information with sufficient time resolution to follow reflectance changes produced by the direct application of 2 sec trains of electrical pulses (20 Hz at 0.5 msec pulse duration) to the cerebral tissue. Within 2-3 sec following onset of electrocortical stimulation, an increase in reflectance became visible at 603-605 nm. Reflectance in this spectral region became progressively greater in intensity (absorption decreased) for approx 6-8 sec and then it declined and returned to baseline within approx 30 sec. Throughout this period, a reflectance peak in the spectral region around 595 nm was apparent which changed only slightly when compared to changes at 605 nm. Comparisons based upon rapid scanning spectrophotometry of blood, perfused whole brain and brain slices demonstrate that the absorption at 595 nm is characteristic of hemoglobin while the 605 nm peak can be ascribed to cyt a,a_3. Such investigations of reflectance changes throughout the visible region of the spectrum provide clear definition to the contribution of hemoglobin to light absorption and demonstrate that the relative contribution of absorption by hemoglobin and mitochondrial respiratory enzymes can be distinguished. These data unequivocably confirm that redox shifts of cyt a,a_3 can be recorded from intact brain in the presence of hemoglobin. The decreased light absorption of this cytochrome in response to electrocortical stimulation signals an oxidative shift which provides additional evidence that cytochrome a,a_3 must be partially reduced under normoxic conditions in "resting" brain. Such a condition implies that mitochondrial status and oxidative metabolism are related to brain functional activity and to oxygen supply in a dynamic rather than a passive manner.

284.14 CEREBRAL CYTOCHROME a,a_3 OXIDATION-REDUCTION RESPONSES DURING HISTOTOXIC AND HYPOXIC HYPOXIA IN BLOODLESS RATS. C.A. Piantadosi* and A.L. Sylvia. Departments of Medicine and Physiology, Duke University Medical Center, Durham, N.C. 27710

Perfluorochemical emulsion (FC-43, Green Cross Corp.,Osaka, Japan)exchange transfusion was used to produce a near bloodless state in barbiturate anesthetized, paralyzed mechanically ventilated rats. Hematocrit levels were reduced to approximately 1% by exchange with 4-5 blood volume equivalents of FC-43 during ventilation on 100% oxygen. Following this procedure,mean $PaO_2$was 408±25mm Hg and mean arterial blood pressure (MAP) averaged 90mm Hg. EEG activity remained normal both during and after exchange transfusion. Cerebral cytochrome a,a_3 oxidation-reduction(redox) state was directly monitored in vivo through the translucent skull by differential reflectance spectrophotometry using the wavelength pair 605-620 nm.

Residual hemoglobin was bound to carbon monoxide by permitting the animal to breath 1.0 to 1.5% CO balance oxygen. The transition from hemoglobin to carboxyhemoglobin was followed at the wavelength pair 577-569 nm. Complete equilibration of CO with hemoglobin required 15-20 min.,thereby preventing oxy-deoxyhemoglobin transitions in the optical field during subsequent studies. Post CO -pre CO difference spectra revealed twin absorbance peaks at 569 and 540 nm consistent with carboxyhemoglobin.

During equilibration with CO, the cytochrome a,a_3 redox level remained steady until the 577-569 nm signal stabilized, at which time a gradual partial reduction of cytochrome a,a_3 occurred. However, this redox change was always less than 10% of the total labile cytochrome signal measured as the difference in optical density at 605-620 nm between steady state 100% oxygen breathing and complete anoxia. Animals remained stable on 1.5% CO-balance oxygen gas mixture for long periods of time without changes in EEG activity. Graded hypoxic hypoxia (F_IO_2 0.90-0.30) or constant intravenous infusion of KCN (0.2-0.3 mg/kg/min) subsequently were used to produce further increases in cytochrome a,a_3 reduction level. Cerebral isoelectricity produced by KCN occurred at approximately 50% cytochrome a,a_3 reduction. EEG silence was achieved at a time when MAP was still well above the autoregulatory threshold and when PaO_2 was greater than 400 mm Hg. Hypoxic hypoxia produced isoelectricity at approximately 40% cytochrome a,a_3 reduction. There was more variability than with histotoxic hypoxia perhaps due to greater fluctuations in cerebral perfusion pressure observed during hypoxic hypoxia. These data indicate close interdependence between cerebral electrical activity and mitochondrial function assessed by changes in the redox state of cytochrome a,a_3.

This work was supported by NIH Grant HL-30100 (ALS).

284.15 RELATIONSHIP BETWEEN OXIDATIVE METABOLIC ACTIVITY OF MITOCHONDRIAL CYTOCHROMES AND LOSS OF NEURONAL EXCITABILITY DURING HYPOXIA IN RAT HIPPOCAMPAL SLICES. T.J. Sick, S.M. Pikarsky*, E.L. Solow* and M. Rosenthal. Dept. of Neurol., Univ. of Miami Sch. of Med., Miami, FL. 33101.

To define causes for loss of neuronal excitability in the central nervous system during hypoxia/anoxia, reduction/oxidation (redox) changes of mitochondrial cytochromes were monitored in slices of rat hippocampus by rapid-scanning spectrophotometry. These slices, 350-400 um in thickness, were prepared from rats decapitated under ether anesthesia and transferred to a recording chamber containing oxygenated artificial cerebrospinal fluid (ACSF). In some cases, the rats were perfused through the heart with cold oxygenated ACSF prior to decapitation to remove hemoglobin from the hippocampus. This facilitated measurements of certain cytochromes (c and b) where light absorption by hemoglobin caused interference. Neuronal excitability was signalled by extracellular field potentials which were recorded in the stratum pyramidale of CA1 in response to electrical stimulation of the Shaffer collaterals. Optical spectra at wavelengths from 400 to 650 nm were recorded during normoxia (superfusion with ACSF oxygenated with 95% O_2 - 5% CO_2) and at intervals following the transition to anoxia (95% N_2 - 5% CO_2) and recovery. Subtraction of spectra taken during normoxia from those taken during hypoxia revealed characteristic absorption peaks of cytochrome $a(+a_3)$, b and c at 605, 565 and 550 nm respectively, and for cytochrome $a_3(+a)$ and c at 445 and 416 nm respectively. Growth of these absorption peaks during the transition to anoxia was followed kinetically by taking spectra at 3 Hz, and comparing changes at peak absorption wavelengths with appropriate reference wavelengths. Shifts toward increased levels of reduction proceeded over a similar timecourse at all peak absorption wavelengths indicating that all mitochondrial enzymes reacted to the loss of oxygen in the same manner. Onset of reductive responses coincided with the onset of loss of neuronal excitability as indicated by disappearance of the population spike recorded in CA1. However, full loss of the population spike occurred earlier than the 2 min required for full reduction of the cytochromes. This is similar to our previous observation that in rat cerebral cortex in vivo, EEG suppression occurred earlier than full reduction of cytochrome a,a_3. These data indicate that the tight coupling between brain oxidative phosphorylation and electrical activity, also occurs in the hippocampal slice, despite earlier reports of depressed metabolic rate in slice preparations. These data also demonstrate that full mitochondrial reduction, and energy failure, is not prerequisite for, and does not regulate, suppression of neuronal excitability during hypoxia/anoxia.

284.16 MERCAPTOPYRUVIC ACID S-METHYLTRANSFERASE ACTIVITY IN THE RAT BRAIN. J. G. Donahue* and D. P. Henry*. (SPON: J. A. DiMicco). Lilly Lab for Clinical Research, Eli Lilly and Co. and Indiana Univ. Sch. of Med., Indianapolis, IN. 46202.

The significance of S-methylation of endogenous thiols by the brain remains unclear. Thiol S-methyltransferase (TMT) and thiopurine S-methyltransferase (TPMT) are S-Adenosyl-L-methionine utilizing methyltransferases associated with the microsomal and soluble fractions, respectively. The naturally occurring thiols cysteine, homocysteine, and glutathione have been found not to be substrates for either enzyme. We report that both the microsomal and soluble form of the rat brain enzyme methylate the alpha-keto analog of cysteine, mercaptopyruvic acid, formed by transamination suggesting a new pathway in cysteine metabolism. It is well known that cysteine and/or its metabolites are severely neurotoxic. Hence, we have examined the distribution of thiol-S-methyltransferase activity in the rat brain using the 40,000 g supernatant. This fraction contained 80% of the total S-methyltransferase activity in brain using thiopyruvic acid as substrate. Activity is expressed as pmole product formed per hr per mg wet weight, (mean ± SE); n = 4.

Brain Region	Activity
Hypothalamus	.515 ± .147
Midbrain	.340 ± .095
Striatum	.322 ± .104
Cerebellum	.214 ± .025
Pituitary	.183 ± .027
Medulla-pons	.121 ± .015
Cortex	.049 ± .008

Thus, mercaptopyruvate S-methylase activity is heterogeneously distributed in the rat brain. We conclude that thiol S-methyltransferase activity is present in rat brain and that the preferred substrate for this enzyme is the endogenous thiol, thiopyruvic acid, formed by the transamination of cysteine. The low levels of S-methyltransferase found in cortex and the previous report that the cortex is most severely affected by cysteine toxicity (Brain Res. 208:167, 1981) suggest that thiopyruvic acid may be the active agent in this syndrome. Further transamination of methylthiopyruvic acid could be a potential metabolic source of S-methylcysteine and S-methylglutathione which has been found in brain tissue.

284.17 THE RELATION BETWEEN PALMITATE FLUX INTO RAT BRAIN AND BLOOD FLOW UNDER NORMOCAPNIA AND HYPERCAPNIA. A.S. Kimes, H. Takei, Y.Z. Ziylan, D.J. Sweeney and S.I. Rapoport. Lab of Neurosciences, GRC, NIA, NIH, Baltimore City Hospitals, Baltimore, Maryland 21224

Palmitate flux into rat brain regions has been shown to be proportional to the regional cerebral metabolic rate for glucose (rCMRglc) in awake rats (Kimes et al., Brain Res., in press) and to decrease with barbiturate anesthesia (Kimes and Rapoport, Neurosci Abst., 8: 1003, 1982). In addition, the age pattern of regional palmitate flux into brains of Fischer-344 rats (Tabata et al., Neurosci. Abst. 9, 1983) is very similar to the pattern of regional cerebral blood flow (rCBF) (Ohata et al., Brain 104: 319-332, 1981). Because oxidative metabolism and blood flow usually are coupled, the proportionality between palmitate flux and rCMRglc could reflect blood flow, if palmitate delivery to brain were limited by blood flow. To examine this issue we measured palmitate flux in brain regions of normocapnic and hypercapnic rats. Administration of 8.5% CO_2 in air increased $PaCO_2$ from a normal 35-45 mm Hg to 70-80 mm Hg, and also doubled rCBF as measured by [14C]iodoantipyrine (Ohno et al. Stroke 10:62-67, 1979).

To measure palmitate flux, femoral arterial and venous catheters were implanted under pentobarbital anesthesia. Rats recovered from anesthesia for 4½ hrs before they were placed into a chamber where they received either 8.5% CO_2 in air or 100% air. Blood gases were measured on arterial blood collected 20 min after the start of the CO_2 or air. After 30 min an i.v. bolus of 450 µCi/kg [14C]palmitate was given and timed arterial blood samples were collected, until the animals were killed 4 hr after injection.

Plasma radioactivity due to [14C]palmitate was determined by extraction and chromatography, and plasma palmitate was determined by gas chromatography. Regional brain radioactivity was determined by quantitative autoradiography on brain sections.

Unidirectional flux was calculated from the following equation: J = kCplasma, where J is flux (µmole/g.sec), k is the transfer constant from plasma to brain (sec^{-1}) and Cplasma equals the plasma cold palmitate concentration. k was determined by dividing brain radioactivity by the integral of plasma [14C]palmitate between 0 and 4 hr. Comparison of mean values for 43 regions of J's and k's showed no statistical differences between a group of 5 normocapnic rats and a group of 7 hypercapnic rats in which hypercapnia had caused a 2-fold increase in rCBF.

We conclude that palmitate flux into the rat brain is not flow limited, as was expected from brain uptake index of 5.8% (Partridge and Mietus, J. Neurochem. 34: 363-466, 1980). Therefore, the proportionality in the brain between palmitate flux, blood flow and oxidative metabolism is due to some other factor. We propose that palmitate flux relates turnover of lipid-containing components of brain structure to oxidative metabolism.

284.18 CEREBRAL CORTEX AND SPINAL CORD IN AMMONIA INTOXICATION. W. Raabe and S. Lin*. Depts. Neurology, VA Medical Center and University of Minnesota, Minneapolis, Minneapolis, MN 55417.

Ammonia intoxication causes an encephalopathy in animals and man. Animal studies suggest that an effect of ammonia on postsynaptic inhibition causes or contributes to the encephalopathy because ammonia intoxication simultaneously affects cerebral cortical postsynaptic inhibition and the EEG. Recent studies showed that ammonia intoxication affects spinal postsynaptic inhibition at the same tissue NH_4^+ concentrations, about 1 µMol/g, which affect cortical postsynaptic inhibition. Spinal but not cerebral cortical postsynaptic inhibition can be studied in man. To investigate whether a dysfunction of spinal inhibition due to increased NH_4^+ in the spinal cord is indicative of an increase of NH_4^+ and a subsequent dysfunction of postsynaptic inhibition in the cerebral cortex, we studied the effect of different degrees of ammonia intoxication on cerebral cortical and spinal NH_4^+ in the same animal. Since ammonia intoxication causes encephalopathy without an energy depletion of the tissue, we included an investigation of energy metabolites in our study.

In adult cats, the cerebral cortex and spinal cord were widely exposed and covered by warm mineral oil pools. Pool temperatures and body temperature were kept at 37.5°C. The cats received either 0, 2, or 4 mMol/Kg ammonium acetate (NH_4Ac) i.v. within 30 min. Thereafter, a blood sample was drawn, the cerebral cortex and spinal cord were simultaneously frozen. Tissues were extracted with perchloric acid and metabolites were enzymatically determined. Plasma NH_4^+ was 0.05, 1.24 and 4.64 µMol/g after 0, 2 or 4 mMol/kg NH_4Ac respectively; cerebral cortical NH_4^+ was 0.05, 0.88 and 4.14 µMol/g; spinal cord NH_4^+ was 0.06, 0.29 and 3.64 µMol/g respectively. In cortex and cord, glutamine and lactate increased with the increase of NH_4^+. Glutamate, ATP, ADP, AMP, the adenylate energy charge, PCr, glucose, α-ketoglutarate, and pyruvate did not change with the different degrees of intoxication.

Our data show that spinal NH_4^+ correlates with cortical NH_4^+, but spinal NH_4^+ always is slightly lower than cortical NH_4^+. A dysfunction of postsynaptic inhibition occurs in cortex and cord at about the same NH_4^+ levels. Therefore, the use of spinal inhibition as an indicator of the occurrence of a dysfunction of cortical inhibition will slightly underestimate the effect of NH_4^+ on cortical inhibition in ammonia intoxication. In addition, we demonstrate that tissue NH_4^+ concentrations of 3-4 µMol/g are not associated with an energy depletion in the cerebral cortex or spinal cord.

285.1 A METHOD PROVIDING RELIABLE MEASUREMENTS OF INTRACRANIAL SELF-STIMULATION IN SPITE OF THE PRESENCE OF CONTINGENT MOTORIC ACTIVITY. P.P. Rompré, L. Phillipe* and E. Miliaressis. School of Psychology, University of Ottawa, Ottawa, Ontario. K1N 6N5

Bar pressing rates for intracranial self-stimulation (ICSS) represent the most convenient as well as the most controversial way for measuring the reinforcement value of the stimulation. For instance, in examining the neural substrates of reward, it was demonstrated that bar pressing rates as often obtained by fixed electrical parameters leads to a variety of mapping conclusions depending on the choice of pulse frequency and intensity (Miliaressis et al, Brain Res. Bull. 8, 693-701, 1982). In an attempt to solve this problem, a psychophysical method has been proposed. The procedure consisted of looking for the number of cathodal pulses required at each brain site in order for the animal to maintain a predetermined ICSS performance. This method provided reliable mapping data with exception of brain sites in which ICSS was accompanied by motoric components. In this case, the overall pulse frequency/responding (F/R) function was depressed leading to slightly different neuronal estimates according to the value of the criterion performance. In the present study, two alternative criteria were studied: the number of presses equal to 1) 50% of the maximum performance (M50) and 2) theoretical zero (θ0) performance inferred by the regression analysis of the linear portion of the F/R function. In order to compare the reliability of the above two criteria, the F/R functions for lateral hypothalamic (LH) ICSS were examined in the rat under the following conditions: A) LH ICSS alone, B) LH ICSS with contingent motoric components of varying severity introduced experimentally by simultaneous stimulation through a second electrode implanted in the fasciculus longitudinalis medialis (FLM). It was observed that the motoric effect led to a depression of the behavioral asymptote accompanied by a reduction of the slope of the F/R function. The above changes were related to the severity of the motoric effect which was controlled by the pulse intensity delivered through the FLM electrode. In contrast, the intersection of the F/R function with the pulse frequency axis (θ0) remained unchanged in all conditions. Reward measurements by the use of M50 procedure were found to be less reliable compared to those inferred from θ0 calculations.

285.2 INTERRESPONSE ANALYSIS OF SPONTANEOUS AND PROVOKED INTRACRANIAL STIMULATION EPISODES. J. A. Wagner*and R. J. Katz . (SPON: T. Sejnowski). Department of Psychology, The Johns Hopkins University, Baltimore, MD 21218.

The episodic behavior of rats given chronic access to reinforcing intracranial stimulation was examined for systematic trends in the times between successive responses (interresponse times; IRTs) by multifactorial statistical analyses. Five systematic trends were found across animals. (1) In spontaneous episodes, initial interresponse times declined in a logarithmic fashion reaching asymptote between the tenth and the fifteenth response. (2) This trend varied with the length of time before the initiation of the episode. Short pre-episode times generated short, asymptotic initial interresponse times. (3) Spontaneous episodes characteristically ended with linearly increasing interresponse times. (4) Responses made during extinction did not exhibit any of the above trends. (5) Provoked episodes began with short and already asymptotic interresponse times. These findings suggest that a priming process cumulates within spontaneous episodes and is already active in spontaneous episodes with short pre-episode times and in provoked episodes. The results also provide evidence that arousal by provocation may be similar behaviorally to priming activation. This work was supported by a Biomedical Research Grant.

285.3 BEHAVIORAL MEASUREMENT OF NEURAL REFRACTORINESS: ESTIMATES OF EARLY RECOVERY USING PULSES OF UNEQUAL INTENSITY. E. Miliaressis, L. Philippe*, A. Durivage*, and P.P Rompré. Ecole de Psychologie, Université d'Ottawa, Ottawa, K1N 6N5 Canada.

The neural substrata of electrically induced behaviours can be characterized functionally using Yeomans' psychophysical version of the double pulse technique (Yeomans, J., Physiol. Behav., 15:593, 1975). However, the early recovery following the absolute refractory period of the fastest conducting neurons is obscured by the presence of residual latent summation which normally occurs at short inter-pulse intervals. This phenomenon is undesirable since a precise estimate of the early recovery is critical for the comparison of neural substrata having similar excitability properties. In a previous study (Miliaressis, E., Physiol. Behav., 26: 709, 1981) we showed that the use of pulses of unequal intensity (the conditioning or C pulse being larger than the test or T pulse) results in a reduction of the latent summation effect. In the present work, the effect of C=T and C>T conditions was systematically examined in intracranial self-stimulation behaviour, using hypothalamic, mesencephalic, thalamic and raphe electrodes.

As predicted, the unequal pulse condition resulted in a decrease of residual latent summation. As a consequence, differences in early recovery between the investigated areas, not seen by the usual C=T procedure, were revealed. The fastest recovery was observed in the raphe region (.35 msec. following application of the C pulse), followed by the ventral mesencephalon, (.4 msec) the lateral hypothalamus, (.45 msec.) and the thalamus, (.7 msec.).

It was concluded that the unequal pulse condition is critical for accurate comparison of the excitability cycles of different neural substrata.

285.4 REFRACTORINESS OF REWARD-RELEVANT NEURONS IN THE NUCLEUS ACCUMBENS AND ADJACENT STRUCTURES. C. Bielajew, S. Walker*, and G. Fouriezos. BSR Lab., Sch. of Psychol., Univ. of Ottawa, Ottawa, Ont., Canada K1N 6N5.

Recently, there has been considerable interest in examining the electrophysiological properties of self-stimulation structures anatomically related to the medial forebrain bundle. Refractory periods have been documented for self-stimulation of the frontal and cingulate corticies (Schenk, S. & Shizgal, P., Physiol. Behav., 28:133, 1982; Silva, L. et al., Soc. Neurosci. Abstr., 8:625, 1982). The range of values obtained (approx. 1.0 to 6 ms) far exceeds that frequently reported for self-stimulation sites located along the trajectory of the medial forebrain bundle, 0.4 to 1.5 ms (Bielajew, C. & Shizgal, P., Brain Res., 237:107, 1982; Bielajew, C., et al., Physiol. Behav., 27:95, 1981; Rompré, P.-P. & Miliaressis, E., ibid., 24:995, 1980), providing indirect evidence that the neural circuitry underlying brain stimulation reward comprises neurons with a wide range of fiber diameter (Swadlow, M. & Waxman, S., Exp. Neurol., 53:128, 1976). The present study was designed to extend these data to include the excitability properties of cells activated by rewarding stimulation of the nucleus accumbens and adjacent structures. Using Yeomans's equal and unequal pulse paradigms (Yeomans, J., Physiol. Behav., 15:593, 1975; Yeomans, J., ibid., 22:911, 1979), we assessed both refractory periods and the separate contributions of the absolute and supernormal phases of excitability in six Long-Evans male rats. With pulses of equal amplitude, recovery from refractoriness began no earlier than .8 ms and ended as late as 6 ms. When the T pulse amplitude was increased to 1.6 times the C pulse no difference in the time course of recovery between the larger T pulse and equal pulse conditions was observed, a finding in agreement with the idea that this wide range of values primarily reflects recovery from absolute refractoriness. In the third procedure, no consistent supernormal period was found as a result of increasing the C pulse amplitude to 1.6 times T. These findings favor the conclusion that the substrate for brain stimulation reward in the region of the nucleus accumbens comprises several populations of neurons with different absolute refractory periods and little or no supernormality. The time course of recovery of these neurons is similar to that reported in other forebrain structures and only slightly overlaps the shorter refractory periods observed in the medial forebrain bundle.

285.5 DISSOCIATION OF MFB SELF-STIMULATION AND EXPLORATION FIBERS USING THE COLLISION TECHNIQUE: A.Durivage* and T.Miliaressis. Behavioural Neurophysiology lab. , School of Psychology, University of Ottawa, Ottawa, Ontario, Canada, K1N-6N5.

Exploratory activity represents one of the most frequent correlates of Intracranial Self-Stimulation (ICSS) in the Medial Forebrain Bundle (MFB). In the rats, the correlation is so good that several investigators use Exploration as a predictor of good self-stimulators during initial screening. In the present work, the possibility that the two behaviours were elicited by the same set of fibers was examined. Seven rats were implanted with two miniature moveable electrodes (Miliaressis,Brain Research Bull. 7(6) : 715-718,1981) each one aimed at the lateral hypothalamic or the ventral mesencephalic level of the MFB. Shizgal's behavioral version of the collision test (Shizgal et al. J. of Comp. and Physiol. Psycho. 94 (2): 227-237,1980) was then applied between the two electrodes, independently for each behaviour. Using this test, a collision effect obtained in one of the two behaviours at the exclusion of the other is considered as an evidence that the two behaviours involve different sets of fibers. In four rats, a collision effect was observed only in ICSS. Two animals showed no collision at all and finally a collision effect in Exploration at the exclusion of ICSS was obtained in a single rat. The conduction velocity estimates as inferred from the collision time were 2 to 5 meters/second for ICSS and 4.5 meters/second for Exploration. Similar conduction velocity estimates for ICSS were found by Bielajew and Shizgal (Brain Research 237,107-119,1982). The data suggest that the presence of ICSS and Exploration by MFB stimulation can be explained by current spread on two different sets of fibers.

285.6 DOES HIPPOCAMPAL SELF-STIMULATION DEPEND ON ELICITATION OF EPILEPTIFORM ACTIVITY ? Kenneth A. Campbell and N. W. Milgram. Dept. of Psychology, University of Toronto, Scarborough Coll., West Hill, Ontario, Canada.

It has been suggested that self-stimulation in certain limbic structures - including the hippocampus - may be dependent on the propagation of epileptiform discharges. Repeated electrical stimulation (kindling) increases epileptiform afterdischarge (AD) triggered by stimulation trains. We have previously reported that the very slow acquisition of responding for hippocampal stimulation in naive rats (8-14 daily 30-min sessions) can be greatly facilitated (to 1-3 sessions) by a prior program of hippocampal kindling (Campbell et al., Brain Research, 159, 458, 1978). The present research tested the premise that hippocampal self-stimulation depends on elicitation of AD by monitoring hippocampal EEG during self-stimulation in 3 studies.

First, hippocampal EEG was recorded in 4 previously-kindled rats and in 2 naive rats during all acquisition sessions for hippocampal self-stimulation. In each rat, AD was always triggered by the first daily hippocampal stimulus (0.5 sec, 30 µA rms). Sometimes a second AD was triggered later in the session. There was no difference in elicitations of AD between kindled and naive rats, even though kindled rats were self-administering over 200 stimulations per session before the naive rats learned the response.

Second, after the 4 kindled rats were maintaining stable rates at 30 µA, self-stimulation current intensity was lowered to 10 µA for 2 sessions. Response rates increased significantly for each animal, while AD was elicited in only one of the 8 sessions sampled.

Third, 5 other rats which maintained stable self-stimulation at 5-10 µA underwent extinction for 4 sessions. When current was again delivered at 5-10 µA, self-stimulation responding recovered without eliciting hippocampal AD.

The present results suggest that it is not necessary to evoke AD to maintain hippocampal self-stimulation. Thus, it appears unlikely that kindling facilitates acquisition of hippocampal self-stimulation by augmenting epileptiform activity. However, the possibility that hippocampal self-stimulation below local AD threshhold may elicit AD elsewhere cannot be excluded on the basis of the present results.

285.7 BRAIN-STIMULATION REWARD: THRESHOLDS VERSUS RESPONSE RATES FROM VARIOUS BRAIN LOCI. M. Payton*, C. Kornetsky and D. Rosene. Laboratory of Behavioral Pharmacology & Department of Anatomy, Boston University School of Medicine, Boston, MA 02118.

The most commonly used dependent measure in intracranial self-stimulation (ICSS) experiments is rate of response. In comparing rates of response elicited by stimulation to different brain loci, high as opposed to low response rates are usually believed to coincide with high versus low sensitivity, respectively. In order to directly determine the relationship between sensitivity and response rate for ICSS we compared the absolute threshold with rate of response in animals with electrodes in one of five different brain loci.

Thirty-five male albino rats (CDF - Charles River Lab.) were implanted with bipolar stainless steel electrodes in either the preoptic area (POA), anterior hypothalamic area (AHA), medial forebrain bundle (MFB), dorsal medial hypothalamus (DMH), or ventral tegmental area (VTA). Reward thresholds were determined using a rate independent discrete trial procedure. After completion of the threshold determinations animals were retrained on an FR2 reinforcement schedule and complete rate-intensity power functions were determined on two consecutive days. The mean intensity of the two highest rates generated was then used as the test intensity in five subsequent testing days. For each animal the mean response rate for the five days was used as the basal rate datum.

A comparison of the two methods indicates that there are significantly greater differences between thresholds than between rates as a function of brain area. The table shows the mean reward thresholds and response rates for animals with electrodes in each of the five brain areas. Rank assignment is from lowest to highest for both threshold and rate. These results indicate that brain areas with the lowest response rate do not necessarily have the highest threshold and vice versa. Thus, caution must be employed when interpreting the relative sensitivity of various brain areas to rewarding brain stimulation based on response rate alone.

Area	N	$\bar{X}$ Threshold (µA)	Rank	$\bar{X}$ Rate (respon/min)	Rank
DMH	5	37.7 ± 6.7*	1	55.6 ± 8.4*	4
AHA	8	68.8 ± 1.2	2	52.5 ± 2.6	2
POA	7	68.9 ± 10.2	3	41.9 ± 2.6	1
VTA	8	81.3 ± 4.1	4	53.7 ± 3.9	3
MFB	7	86.5 ± 6.0	5	62.8 ± 7.9	5

* Standard Error of the Mean

(Supported in part by NIDA grant DA 02326 and NIH grant NS 19416).

285.8 APOMORPHINE RAISES BRAIN STIMULATION REWARD THRESHOLDS. G. Fouriezos and S. Francis*. BSR Lab., Sch. of Psychol., Univ. of Ottawa, Ottawa, Ont., Canada K1N 6N5.

The effects of apomorphine, a direct agonist of dopamine receptors, on rates of response for rewarding brain stimulation have been reported to be facilitatory (Wauquier, A. & Niemegeers, C.J.E., Psychopharmacol. 30:163, 1973), inhibitory (St. Laurent, J. et al., Pharmacol. Biochem. Behav., 1:581, (1973), and without consistent effect (Broekkamp, C.L.E. & van Rossum, J.M., Psychopharmacol., 34:71, 1974). With the possibility in mind that apomorphine's influence might be rate-, time-, and dose-dependent we documented its action on rewarding brain stimulation thresholds over a two hour period following intraperitoneally administered doses of 0, .01, .03, .1, .3, and 1 mg/kg.

Four Long-Evans male rats prepared with chronic lateral hypothalamic electrodes were trained to earn 0.3 s trains of 0.1 ms cathodal pulses in a paradigm specifically designed for the rapid collection of threshold data (Fouriezos, G. & Nawiesniak, E., Soc. Neurosci. Abstr., 8:624, 1982). Briefly, the rats worked through a sequence of pulse frequencies that began at 100 Hz and dropped in 20% steps with every 8 earned stimulations. Current levels were selected so that rats would continue responding for about 30 s. Resets to the starting frequency occurred at 1 min intervals under timer control. Thresholds were defined as the frequencies of stimulation interpolated to support half-maximal rates of response. Drug tests followed randomized orders and they were separated by at least three days. The rats were injected after five threshold determinations and tests continued at five min intervals for two hours post-injection.

Low doses (.01-.1 mg/kg) produced dose-related increases in frequency thresholds that began within 10 min post-injection and lasted from 20 to about 90 min respectively. The effects of the two higher doses (.3 & 1 mg/kg) were difficult to discern because these doses produced stereotypical perseveration of lever pressing; threshold determinations were precluded by the rats' failure to quit responding. (When stereotypy was witnessed, turning the stimulation off completely failed to alter the persistent responding.)

With the injections of apomorphine conceived as providing non-contingent stimulation of one critical level of reward circuitry, the raised thresholds are viewed as reflecting an increased response-contingent "signal" required by the elevated background "noise". If this interpretation is correct, then these data provide strong evidence supporting a role for dopamine elements as important components of brain stimulation reward circuitry.

286.1 STIMULATION INDUCED BEHAVIOR IN NORMAL AND METHYLAZOXYMETHANOL (MAM) TREATED INFANT RATS. T.H. Moran*, P.R. Sanberg and J.T. Coyle (SPON: J. Wirth). Depts. of Psychiatry and Neuroscience, Div. of Child Psychiatry, Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

Rat pups, 10 days of age and younger, become behaviorally activated by short trains (500 msec) of medial forebrain bundle (MFB) electrical stimulation, emitting a series of behavioral responses that include mouthing, licking, pawing stretch and lordosis responses. As development progresses from Day 3 to Day 10, the responses become more organized. Day 15 and older pups, however, do not respond to these stimulation parameters (Moran et al. J. Neurosci. 3, 10, 1983). In an attempt to identify a neural basis for the increasing behavioral organization with age and the absence of behavioral activation at Day 15, we compared the responses to stimulation of pups whose mothers had been treated at gestational day 15 with saline or the antimitotic agent methylazoxymethanol acetate (MAM) which produces a severe and lasting hypoplasia of cortical neurons resulting in a greater than 50% decrease in cortical mass (Johnston and Coyle, J. Neurochem. 34:1429, 1980).

Monopolar stainless steel electrodes were aimed at the MFB at the level of the lateral hypothalamus on the morning of testing in 3, 10 and 15-day-old saline or MAM treated pups. Their behavioral responses were observed over three 6 minute periods during which stimulation trains were administered at the rate of 0, 2, or 3 per minute during the first half of each period. The occurrence of all behaviors was recorded every 10 sec.

Behavior increased with increasing frequency of stimulation at ages 3 and 10. Ten-day-old MAM treated pups evidenced significantly less behavioral organization in their responses to stimulation than saline controls, as measured by the temporal ordering of responses. Despite no difference in body weight, MAM, unlike saline pups, continued to respond to stimulation at Day 15, emitting the full range of behavioral responses seen in younger animals. These results suggest a role for the developing cortex in behavioral organization, since cortical hypoplasia prevents the normal responses to the activation of MFB stimulation. The onset of cortical inhibitory mechanisms may channel behaviors in normally developing infant rats and prevent the characteristic activational responses to brief stimulation in Day 15 pups.

286.2 PREOPTIC AREA CONNECTIVITY RELEVANT FOR MATERNAL BEHAVIOR IN THE RAT. M. Numan, J.I. Morrell and D.W. Pfaff. The Rockefeller University, New York, NY 10021.

Lesion studies have indicated that the lateral efferent projections of the medial preoptic area (MPOA) are essential for maternal behavior in the rat (Numan, M. and Callahan, E.C., Physiol. Behav., 25: 653, 1980) and have also suggested that the preoptic region interacts with the midbrain tegmentum in the control of maternal behavior (Numan, M. and Nagle, D.S., Behav. Neurosci., 97: 120, 1983). The present experiment provides neuroanatomical evidence concerning the question of whether MPOA efferents which descend to the lower brainstem via the lateral hypothalamus (LH) are important for maternal behavior.

Fully maternal lactating rats (3 days postpartum) received bilateral coronal knife cuts through either the dorsal or ventral LH at the level of the ventromedial nucleus. Control cuts consisted of extending the blade of the wire knife into either the dorsal or ventral LH, producing only minimal damage. The blade of the wire knife was coated with horseradish peroxidase (HRP), obtained by allowing a 2 µl drop of a 4% solution of HRP in water to dry on the blade. The maternal behavior of all females was studied for four days postoperatively, at which time the females were perfused with glutaraldehyde/paraformaldehyde. Frozen 50 µm sections of the brain tissue were subsequently processed for the localization of retrogradely filled HRP cell bodies using tetramethyl benzidine as a chromagen. Our analysis focused on those regions thought to be important for maternal behavior.

The ventral LH cuts retrogradely filled more MPOA cell bodies with HRP than did the dorsal cuts and the dorsal cuts filled more lateral preoptic area (LPOA) cell bodies with HRP than did the ventral cuts. Females which received control cuts showed a similar pattern, but fewer cells were retrogradely filled. The ventral cuts did not disrupt maternal behavior, suggesting that MPOA axons which descend directly to the brainstem via the ventral LH are not essential for maternal behavior. The dorsal cuts did disrupt maternal behavior, suggesting the importance of an MPOA-to-LPOA-to-brainstem circuit for maternal behavior. Finally, the dorsal cuts also labeled more cells in the ventral tegmental area (VTA) and the bed nucleus of the stria terminalis than did the ventral cuts, while the latter tended to retrogradely fill more cells in the diagonal band-septal region. These data also support the hypothesis that ascending VTA fibers may have a role in the regulation of maternal behavior. (Supported by USPHS grants HD06377 & HD16327)

286.3 MEASUREMENT OF SEXUAL MOTIVATION: SECOND ORDER SCHEDULES OF PRESENTATION OF RECEPTIVE FEMALE. P.J. Fray*, E.M. Kostarczyk*, S.J. Taylor* and B.J. Everitt* (SPON: European Neuroscience Association). Univ. of Cambridge, Dept. of Anatomy, Downing St., Cambridge CB2 3DY, England.

If a male rat could be trained to work for a prolonged period for a female, the vigour of responding before the female is presented could be used as an index of sexual motivation.

In Experiment I, eight hooded rats were trained to respond on each of two levers for food on a FR10 schedule. At a different time of day, a light and a white noise were preconditioned to a receptive female during 20 pairs of sessions. In one session, one of the stimuli (CS+) was presented after a random time and followed by the female; in the other session, the other stimulus (CS-) was presented alone. Thereafter, both levers were present. Each time the rat made 10 responses on one lever the CS- was turned on for 1 sec. After 10 responses on the other lever the CS+ was turned on for 1 sec, and when a predetermined number of CS+ had been earned the CS+ was kept on for a further 29 sec and a receptive female was immediately introduced. The session ended 2 min after ejaculation. The number of CS+ required to obtain the female was increased from 1 until the rats were taking about 15 min to earn her. They were then switched to a 2nd order FI 15-min (FR10:S) in which the female was delivered with the first CS+ to be earned after 15 min had elapsed. The rats quickly learned to discriminate between the levers, with 80% of the stimuli earned being CS+ by the 5th session. The rats for which CS+ was light learned faster than those for which CS+ was noise. When the correct lever was at the front of the chamber the rats also learned faster. The female was presented from the front, and the rats were observed to spend more time there. A characteristic FR pattern of responding developed for the CS+, and a typical FI scallop over the whole session.

In Experiment II, another group of rats did not undergo the preconditioning, the female was held in a small box on the roof which opened into the operant chamber, and the rats were maintained on a FR(FR) schedule. They learned to discriminate as fast as the other rats, indicating the lack of need for the preconditioning. The light was learned about more quickly than the noise as before, but there was now no effect of the position of the correct lever. A characteristic FR pattern developed both for the CS+ and over the session as a whole.

This technique successfully produced a baseline of responding on which the effects of hormones, drugs or lesions can be tested acutely. The observed effects should be attributable to changes in sexual motivation, uncontaminated by effects on copulatory ability, since they would be evident before the female was presented for the first time.

286.4 AGGRESSION AND HOMOSEXUAL BEHAVIOR IN FEEDLOT STEERS. W. R. Klemm, L. M. Schake*, and R. F. Sis*. Depts. of Veterinary Anatomy & Animal Science, Texas A&M Univ., College Station, Tx. 77843.

A common male homosexual vice in feedlot cattle that are implanted with growth promotants is an activity wherein some steers ("bullers"), sexually attract other steers ("riders"). A leading theory to explain this behavior is that bullers are presumed to be excessively feminized and give off sexually stimulating pheromones.

We studied this issue in a West Texas commercial feedlot by monitoring the behavior of 4 pens of 160 cattle each. Because pheromones are detected in cattle by the vomeronasal organ, we tested the pheromone hypothesis by cauterizing duct openings that lead to the vomeronasal organ. Cautery of riders statistically reduced bulling behavior, but the magnitude was still large. Cautery of bullers revealed that they were subjected to less mounting than were the non-cauterized bullers in the same pen.

We believe that a more basic cause of bulling is aggression. Our observations to support this idea include the following: 1) most (over 80%) of all steers in a pen did not engage in social contesting as did both bullers and riders; 2) riders were overtly aggresssive, mounting the bullers repeatedly to the point of injury and exhaustion; 3) bullers themselves were overtly aggressive, in that they commonly mounted riders and other bullers, and in addition had conventional aggression scores about twice that of riders; and 4) the amount of bulling was much greater during the periods of greatest social stress (just after the herds were formed and, in one pen, just after 50 new steers were added to the pen).

The physiological mechanisms of this behavior remain to be discovered. Most of the published theories are based on inconsistent or conflicting evidence. We are currently conducting karyotypic analysis on chromosomes of bullers and riders. Motion picture films (samples of which will be shown in the session) are being examined for visual cues which may stimulate the riders. Whatever the mechanisms, we offer a new hypothesis about bulling; namely that it is a ritualized "game" based on hierarchy contesting.

(This research was supported by the Texas Cattle Feeders Assoc., Amarillo, Tx.).

286.5 A CHOLINERGIC COMMAND CIRCUIT FOR SEPARATION DISTRESS? J. Panksepp*, L. Normansell*, S. Siviy*, A. Buchanan*, A. Zolovick*, J. Rossi and R. Conner*. (SPON: F.G. DeEskinazi) Dept. of Psychology, Bowling Green State University, Bowling Green, OH 43403

Past work analyzing neurochemical controls of isolation induced distress vocalizations (DVs) has not identified a neurochemical system which drives DVs (Panksepp, et al., Pharmac. Biochem. Behav., 1980, 12, 437 & 1980, 13, 673). We have now found that curare, the prototypical neuromuscular nicotinic-cholinergic receptor antagonist, administered into the fourth ventricle region of young chicks produces a stereotyped hyperemotional behavior pattern characterized by rapid head scanning, vigorous flight behaviors, and repetitive DVs. Increased DVs were most apparent in animals tested in the presence of other chicks or mirrors--social stimuli which typcially reduce DV rates markedly. The effect was clearly apparent at 0.5 to 1.0 ug/bird, while at 5 and 10 ug doses, motor discoordination accompanied the highly agitated emotional state. Since neither central nor peripheral mecamylamine simulated a curare-like emotional state, it is questionable whether the observed effect was simply due to nicotinic receptor blockade. The possibility that curare is a cholinergic agonist centrally, was supported by the ability of carbachol (1 ug) to simulate curare's fear-like agitation (but DVs were absent), however both mecamylamine and scopolomine (10 ug, i.c.v) failed to block curare-induced DVs. Curare's effect (at 1 ug) was also not modified by intraventricular morphine (5ug) or nicotine (25nMoles), which themselves reduced DVs. The curare effect was not simulated by arecoline (1 mg/kg), physostigmine (0.5 mg/kg) or pilocarpine (5 mg/kg) given i.p.

In summary, the curare induced emotional state implicates cholinergic synapses in direct activation of DVs, but it remains unclear whether the effect is due to activation or inhibition of nicotinic or some other receptors. The ambiguities encountered may be due to the presence of several cholinergic emotive command circuits in the brain stem. For instance, fear and separation-distress systems may both be coded via nicotinic circuits, which, in the normally functioning brain, exhibit reciprocal functional antagonism.

286.6 OLFACTORY INFLUENCES ON THE INGESTIVE BEHAVIOR OF INFANT RATS. Leslie M. Terry* and Ingrid B. Johanson. Dept. of Psychology, Florida Atlantic Univ., Boca Raton, FL 33431.

Young, deprived rats will respond to oral infusions of milk with an impressive behavioral activation (1). Typically, activation accompanies ingestion of large volumes of the milk and consists of mouthing, probing, and vigorous locomotion. Furthermore, specific components of this activation can come to be elicited by a neutral odor after repeated pairing with infusions of milk (2). Together, these findings suggest that the odor of milk might similarly elicit some components of this behavioral activation.

Litters of 1-, 3-, 6-, 9-, and 12-day-old rats were removed from their mothers 24 hrs before testing. Each litter consisted of 5 pairs of pups. Within each pair, one pup was presented with an airstream scented with the odor of milk and the other pup was presented with an unscented airstream. One of the 5 pairs received no infusion and other 4 pairs received intraoral infusions water, 5% sucrose, .025% quinine hydrochloride, or milk at the same time the odor was presented. Odor alone or odor and infusion were presented for 15 sec every 2 min, for 5 trials. Activity, mouthing, and probing were scored and intake was measured by weighing pups before and after testing.

Three-, 6-, and 9-day-old pups responded to milk odor by becoming active and probing vigorously, both when milk odor was presented alone and when accompanied by infusions of water, sucrose, or quinine. In fact, in some cases milk odor alone elicited as much activity and probing as an infusion of milk. When milk was the solution infused, the presentation of additional milk odor had no effect on the pups' behaviors. This reactivity to milk odor was not observed in 1-day-old pups, and disappeared by 12 days of age. Enhancement of intake by milk odor was also dependent on the age of the pup (with the greatest effect at 9 days of age), as well as the solution infused. At all ages tested, mouthing was a function of the solution infused and was not influenced by milk odor.

These data demonstrate that olfactory cues play a major role in young rats' feeding behavior. Specifically, the odor of milk comes to elicit activity (especially probing) and to enhance intake of infused fluids, perhaps as a result of the pups' experiences with their lactating dam in the first few days after birth.

(1) Hall, W.G. J. Comp. Physiol. Psychol., 1979, 93, 977-1000.

(2) Johanson, I.B., Hall, W.G., & Polefrone, J.M., ms. submitted.

Supported by NICHD Grant HD 16712 to I.B. Johanson.

286.7 ADRENERGIC BASIS OF INCENTIVE MOTIVATION EVIDENCE FROM NEUROTOXIC LESIONS, R.J. Katz and J. Watson*, Department of Psychology, The Johns Hopkins University, Baltimore, MD 21218.

Despite extensive physiological, pharmacologica, biochemical and anatomic studies, and the role of central noradrenaline (NA) in behavior remains uncertain. Upon several grounds including brain self stimulation, and drug self administration, a role in reinforcement is tenable. Saccharin licking is a relatively pure but underinvestigated model of incentive motivation, and may represent a useful means of further validating reinforcement hypotheses generated in other paradigms.

Adult male Sprague-Dawley rats were individually maintained under standard laboratory conditions with food constantly available. Rats were chronically exposed to 2 liquid sources (tap water; 1.0% saccharin in tap water) counterbalanced across subjects and days. Following the establishment of stable consumption patterns rats were injected with the neurotoxin DSP-4(N-2-Chloroethyl-N-ethyl-2-bromo benzylamine HCl; Astra: at 50, 100 mg/kg i.p.in 0.9% saline). Brain norepinephrine was selectively depleted and normally high (>) 80%) choice of saccharin was reduced (<<10%). The reversal occurred within 24 h and persisted for 7 days. A partial recovery of choice occurred within weeks 2-3, postinjection. However, rats never regained stable preinjection patterns of saccharin consumption. A role for noradrenaline in saccharin licking is supported, and this is consistent with findings from other paradigms.

Supported in part by a biomedical research grant from the Johns Hopkins University.

286.8 FIRING VARIATIONS OF AMYGDALOID NEURONS IN RESPONSE TO COMPLEX VISUAL STIMULI DURING OPERANT FEEDING BEHAVIOR OF THE MONKEY. T. Ono, M. Fukuda*, H. Nishino* and K. Sasaki*. Dept. of Physiol., Toyama Med. & Pharmaceu. Univ., Toyama 930-01, JAPAN.

To elucidate amygdaloid (AM) functional roles in the integration of complex visual stimulus processing and the development of emotion, unit activity variation of the AM in response to complex visual stimuli were investigated in feeding situations. Three monkeys (Macaca fuscata) were used. They sat in front of a panel which has a window covered with two shutters, and a bar. A food or non-food material was placed on a turn- table behind the shutters within the monkey's reach. The visual discrimination and feeding task consisted of three stages: 1) visual discrimination of object materials (food, non-food, aversive object etc.) after the first shutter (opaque) opened and before bar-pressing, 2) bar-pressing to open the second shutter (transparent) and 3) ingestion after the second shutter was opened by the last bar press. Among 125 basolateral AM neurons tested, 23 (20%) responded to visual stimuli independent of the nature of the visual objects when the opaque shutter was opened. One fourth responded selectively, with a latency of 100-180 msec, at the sight of food (reward stimuli). Excitation response was predominant. Firing returned to the control level during bar-pressing but increased again during ingestion. In the anterolateral nucleus of the AM, activity was recorded from 337 neurons, and 21 (6%) responded to visual stimuli. There were three characteristic features in the response of neurons in this area of the AM. 1) In contrast to the basolateral area, neurons in the anterolateral AM responded predominantly non-food, especially to aversive materials such as syringe, glove etc. 2) The response decreased or disappeared in repeated (2-10 times) trials (habituation) and 3) The response elicited at the sight of an aversive object such as a syringe disappeared for simultaneous presentation of aversive (syringe) and a small reward objects (raisin, or cookie).

These data suggest functional heterogeneity among the subnuclei of the AM: the basolateral part is concerned predominantly with food (reward) recognition and the anterolateral part is concerned with recognition of aversive materials. The cooperation of these two nuclei might be important in evaluating the meaning of external visual stimuli that forms the basis of the development of emotion.

286.9 BILATERAL AMYGDALECTOMY ELIMINATES CONSUMMATORY NEGATIVE CONTRAST. H. C. Becker*, M. F. Jarvis*, G. C. Wagner, and C. F. Flaherty. Psychology Department, Rutgers University, New Brunswick, NJ 08903.

The consummatory behavior of rats shifted from a 32% to a 4% sucrose solution declines to a level substantially below that of unshifted animals that have only experienced the 4% solution (a negative contrast effect-NCE). This decrement in performance has been attributed, at least in part, to emotional responses (Flaherty, C.F., Anim. Learn. Behav., 10:409, 1982). Possible limbic system involvement in contrast has not been extensively investigated. Earlier studies have found that NCE are influenced by lesions of the septum or hippocampus (Flaherty, C.F. et al., Physiol. Behav., 6:431, 1971; Kramarcy, N. et al., Physiol. Psych., 1:248, 1973). The effects of lesions of the amygdaloid nucleus on NCE are evaluated in this study.

Twenty rats were divided into shifted and unshifted groups. Shifted rats received 5 min access to 32% sucrose for 10 days and then 4% sucrose for 4 additional postshift days. Unshifted rats were maintained on the 4% solution for all 14 days. Two weeks prior to testing, half of these groups (shifted and unshifted) received amygdala lesions (2mA for 20 sec). The remaining animals received sham operations.

Shifted animals that received sham lesions exhibited a reliable NCE on the first 2 postshift days. However, NCE did not occur in shifted amygdalectomized rats. This was supported by the significant interaction sucrose x lesion x day (F (4/56) = 2.88, p .05). Subsequent Fisher's LSD tests (p .05) indicated unshifted shams licked reliably greater on the first 2 postshift days than shifted shams and the shifted lesioned rats licked reliably greater on postshift days 1 and 2 than shifted sham rats.

Given that the amygdala is (a) anatomically and physiologically linked to the taste system, (b) involved in food and water intake, and (c) involved in learned modifications of food intake (e.g. conditioned aversions), it is suggested that this neural structure may play a role in reward evaluation, and in particular, incentive relativity.

286.10 EFFECTS OF ANTEROMEDIAL, SUPRA-RHINAL AND COMBINED PREFRONTAL LESIONS ON FIXED INTERVAL RESPONDING IN THE RAT. E. A. Murry. Dept. of Psychol., Univ. of Georgia, Athens, GA 30602.

The function of the prefrontal cortex has been conceptualized as serving an important role in the temporal structuring of behavior. Theoretical suggestion and experimental data offer indirect support for the involvement of inhibitory processes in situations which require the temporal regulation of behavior. As animals with prefrontal damage exhibit behavioral disinhibition in a variety of conditions, the prefrontal cortex has been thought to have important inhibitory functions. Signs of disinhibition have been most consistently found with lesions of the orbital prefrontal cortex in primates. Previous studies assessing the effects of prefrontal lesions on differential reinforcement of low rates schedules (DRL) among various species have offered controverted results. Behavioral disinhibition may also be measured with the use of a fixed interval schedule (FI), in which a characteristic (scalloped) patterning of responses occurs. Previous research has shown that orbital, but not dorsolateral prefrontal lesioned monkeys exhibit elevated response rates and an alteration in the normal FI scallop.

In order to determine whether the anteromedial and supra-rhinal prefrontal cortices in the rat may be differentiated in terms of FI performance, 20 rats were water deprived and trained on a FI 80 sec. schedule (water reinforcement) prior to administering anteromedial, supra-rhinal or combined prefrontal aspiration ablations (5 per group; sham control). The animals were allowed 5 days recovery prior to post-operative testing.

Data indicate that the anteromedial group retains the basic FI scallop with a significant increase in response rate. The supra-rhinal group showed a significant increase in response rate with a concomitant alteration in the scallop such that the number of responses emitted during earlier segments of the inter-reinforcement interval was increased. Similar changes were seen in the combined lesion group without a significant increase in response rate. Sham operated animals showed a decrease in response rates relative to pre-operative rates. All groups exhibited responding comparable to that of pre-operative performance by the seventh day of post-operative testing.

The characteristic post-operative adipsia seen in rats with supra-rhinal (sulcal) prefrontal lesions was attenuated and in some cases eliminated by the pre-operative water deprivation. Subsequent adjunctive supra-rhinal ablations were performed on animals never deprived of water. Pre-operatively water-deprived rats show a relative weight gain and lack of adipsia as compared to these control animals.

286.11 A DIAZEPAM-SENSITIVE STIMULATION-BOUND FEEDING SYSTEM INHIBITS THE AVERSIVENESS IN NUCLEUS GIGANTOCELLULARIS STIMULATION AND LATERAL HYPOTHALAMIC REWARD-ESCAPE. P. E. Simson and E. E. Coons*. Psychology Dept., New York Univ., New York, NY 10003.

Rats that leverpress for brief trains of current to the lateral hypothalamus (LH) but also press to terminate continuous trains show classical reward-escape. Rats in which the same current elicits stimulation-bound feeding (SBF) will not terminate continuous trains except at extremely high currents (Coons, 1964; Simson & Coons, 1982). They show pure reward. Paralleling these associations of SBF and nonSBF with pure reward and reward-escape, respectively, is another finding. LH stimulation reduces rates of pressing for 3-sec escapes from aversion-implicated nucleus gigantocellularis (NGC) stimulation (Carr & Coons, 1982) only if the LH stimulation supports SBF (Simson & Coons, 1982). Otherwise, LH stimulation increases NGC escape.

Evidently, 1) associated with the approach system in LH self-reward is an aversion system supporting withdrawal, and 2) some mechanism primarily associated with SBF can inhibit this and other aversion systems. To better delineate these systems and their interactions, and given that different neural populations may respond differentially to current and pulse frequency manipulations, we examined the effects of varied LH currents and pulse frequencies on NGC escape rates. In nonSBF rats pressing rates to obtain 3-sec escapes from NGC stimulation increased as LH currents increased (pulse frequency constant) and as LH frequencies increased (current constant). By contrast, in SBF rats rates to escape NGC stimulation decreased as LH current increased. However, as pulse frequency increased, the rate pattern of escape from NGC stimulation was U-shaped. This suggests that SBF rats possess an aversion system as do nonSBF rats but one which can only break away from the inhibitory dominance of the approach system at high pulse rates of driving that cannot be compensated for by the increased current-spread recruitment of more of the approach system.

To further characterize the differentially greater association of aversion inhibition with LH reward sites yielding SBF, we tested how diazepam, a potent anxiolytic causing spontaneous feeding in rats, interacts with the effects of SBF and nonSBF stimulation on NGC escape. Diazepam (2.5 mg/kg, i.p.) markedly facilitated the ability of SBF-site stimulation to decrease escape rates from NGC stimulation while having no such effect in non-feeders. Apparently, the aversion-inhibition system is diazepam sensitive and uniquely associated with the approach mechanisms motivating SBF. This reinforces the findings (Carr & Coons, 1982) that an appetitive system is intimately associated with the amelioration of NGC-induced aversion by LH stimulation.

286.12 POTENTIATION OF REWARD BY HUNGER IS OPIOID MEDIATED. K. Carr and E. Simon. Depts. of Psychiatry and Pharmacology, New York Univ. Med. Ctr., New York 10016.

We have previously reported that feeding induced by electrical stimulation in the lateral hypothalamus (LH) is inhibited by naloxone but not its quaternary analogue (K. Carr & E. Simon, Neuropharmacology, 22:127, 1983). It was found that frequency threshold for stimulation-induced feeding (SIF) after naloxone administration increases markedly over the course of a 10 min session of discrete trials. This suggests that naloxone interacts with a post-ingestive event to inhibit further intake, perhaps by increasing the gain in a satiety mechanism or reducing the reward to be obtained from eating during stimulation. Self-stimulation (SS) in LH sites that sustain SIF is known to be controlled by the same factors that control feeding (B. Hoebel, Fed. Proc., 38:2454, 1979) and was therefore used in the present study to further evaluate the interaction between naloxone and the feeding system.

Six rats with electrodes in the LH that supported SS, as well as SIF, were tested. With stimulation intensity held constant, frequency threshold for eliciting 20 SS responses (1-sec trains)/min was determined by the method of limits. When rats were injected with naloxone (2.0 mg/kg, s.c. - a dose that profoundly inhibits SIF) and retested 20 min later, the change in SS threshold (+3.7% ±1.0) did not differ from that produced by vehicle (+0.8% ±2.4).

If naloxone inhibits SIF by facilitating satiety, SS threshold should be elevated in rats that have just completed an SIF session preceded by naloxone injection; this reasoning is based on classic findings that satiety manipulations inhibit LH SS. It was found that naloxone produced the usual progressive elevation of SIF threshold over the course of feeding trials but the change in SS threshold determined immediately thereafter (+6.5% ±2.6) did not differ from when vehicle injection preceded the SIF session (-0.5% ±2.0). The marked elevation of SIF threshold by naloxone may not be due to an exaggerated satiety response.

Food deprivation is known to potentiate LH SS. Rats were therefore deprived for 24 hr. This produced a 16.5% (±1.6) reduction in SS threshold ($p<.01$). Naloxone reversed this effect, producing a 15.1% (±2.0) increase in threshold ($p<.01$).

These results indicate that potentiation of SS reward by food deprivation is mediated by endogenous opioids. Moreover, they prompt speculation that an opioid mechanism is involved in the central representation of the hunger drive state or in enhancing the reward value of food as a function of hunger. The latter seems more compatible with our observations on SIF referred to above. (Supported by NIH MH 35976 and NIH BRSG S07 RR05399-21 to K.C. and NIDA grant DA-00017 to E.S.)

286.13 NEUROLEPTICS DO NOT BLOCK THE REINFORCING EFFECTS OF OPIATES IN THE CONDITIONED PLACE PREFERENCE PARADIGM. W.B. Mackey* and D. van der Kooy (SPON: J.S. Brandes) Neurobiology Research Group, Department of Anatomy, University of Toronto, Toronto, Canada.

The rewarding effects produced by many psychoactive drugs (such as cocaine, amphetamine and apomorphine) can be greatly altered by manipulation of dopaminergic systems within the brain. Dopaminergic involvement is less clearly implicated, however, in the reinforcing effects of other psychoactive drugs, in particular the opioids. Some investigators have reported neuroleptic blockade of opiate reward but others have failed to demonstrate this. To test the hypothesis that dopamine is critical in opiate reward mechanisms, we employed a paradigm involving the demonstration of a "conditioned place preference" for an environment paired with subcutaneous morphine in rats and we investigated the blockade of the place preference by pretreatment with α-flupenthixol, a dopamine receptor antagonist.

The place preference paradigm used involved pairing morphine with one environment and saline with another distinctly different environment alternately for a total of six days. Each pairing lasted 30 minutes. After the pairings each rat was tested in a single, larger test box. Each end of this box was identical to the pairing environments with a smaller neutral area between. Time spent in each end of the test box was recorded over a 10 minute period. The injection order and the environment to be paired with morphine were counterbalanced in this experiment, unlike other variations of the paradigm where the drug in question is paired with a pre-determined, least-prefered environment.

Two concentrations of morphine (1 mg/kg and 5 mg/kg s.c.) and one of α-flupenthixol (.8 mg/kg i.p.) were used. Morphine was administered immediately before the rat was placed in the training box and α-flupenthixol 2.5 hours before the morphine, a length of time shown by others to result in maximum effectiveness. We found no significant differences between the α-flupenthixol pretreated and control (saline pretreated) rats as all groups showed a significant place preference for the environment paired with morphine. A group of rats pretreated with α-flupenthixol but given saline s.c. instead of morphine showed no preference for one environment over the other but did show the characteristic severe immobility and catelepsy of this high dose of neuroleptic. These results suggest little role for dopamine systems in morphine reward. We are presently looking at the effects of other neuroleptics on opiate induced place preference. Positive results by some other investigators attempting to block opiate reward with neuroleptics using the place preference paradigm may have been due to differences between the respective place conditioning procedures used.

286.14 MESIAL PREFRONTAL CORTICAL LESIONS IN RATS ENHANCE TIMIDITY IN SEMI-NATURAL ENVIRONMENTS. R. R. Holson * (SPON: O. Smith), Dept. of Psychology, University of Washington, Seattle, WA 98195

In an earlier report (Holson, Neurosci Abstracts, 1982, 8, pg. 626) it was noted that rats with mesial prefrontal cortical (MFC) lesions evidenced an increased aversion to fear-provoking stimuli across a variety of laboratory situations. It is important to know whether this affective hyperreactivity is an important component of the MFC syndrome, or simply a laboratory curiosity. Hence the present series of experiments was designed to assess risk-taking in MFC rats in a semi-natural environment.

In 2 separate experiments, adult (130 days old) male hooded rats were given discrete electrolytic lesions limited to area 32, and compared postoperatively to unoperated controls in a large (71.3 m^2), unheated environment equipped with numerous dark, enclosed nest boxes (each box 0.22 m^2). Subjects spent more than a month in this environment, which in the first experiment also included adult estrous females. During their stay, rats were fed in different locations daily (lab pellets, usually 30 g. per rat/day), with all pellets placed in a single pile.

Behavior across the 2 experiments was highly similar. Basically, the MFC rats spent more time than controls hiding inside the nest boxes. In both experiments, the lesioned subjects were significantly less likely to participate in the daily food-hoarding sessions at feeding time, were found inside the nest boxes more frequently during daily checks of these boxes, and did not change nest boxes as often as controls. Social dominance was evaluated by recording agonistic encounters in the first experiment, and was lower in MFC rats. Social rank correlated highly with level of participation in the daily food hoarding. In this experiment, body weight of controls dropped significantly more than for MFC rats over the first 2 weeks, presumably due to the greater activity of these animals. In the second experiment, MFC rats displayed lower hoarding and reduced activity outside of feeding periods when caged with other MFC rats or together with controls. Over time, hoarding improved when competing with other MFC rats, but not in the larger MFC/control groups.

It is concluded that, unlike in the laboratory, MFC rats are actually hypoactive in large semi-natural environments with provision of burrow-like hiding places. This lowering of activity, together with low social rank and poor competition for food, suggests that the MFC timidity seen in the laboratory is indeed a primary MFC deficit, one that is important for an understanding of the evolutionary role of prefrontal cortex.

286.15 RATS IN WHICH STRIATAL DOPAMINE AGONISM ELICITS MORE ORAL BEHAVIOR CONSUME MORE DURING LATERAL HYPOTHALAMIC ELECTRICAL STIMULATION. S.E. Bachus & E.S. Valenstein. Psych., U. Mich. Ann Arbor MI 48109

Variability in behavior evoked by electrical stimulation within lateral hypothalamus (ESLH), which is not accounted for by subtle differences in electrode position, is correlated with direction of stereotypy toward external stimuli in response to i.p. amphetamine (Bachus & Valenstein, Neurosci Abst 7:875, 1981). Both amphetamine stereotypy and ESLH-consumption are disrupted by haloperidol (Pijnenburg et al., Psychopharm 45:65, 1975; Phillips & Nikaido, Nature 258:750, 1975) or striatal 6-OHDA (Asher & Aghajanian Brain Res 82:1,1974; Mittleman & Valenstein, Neurosci Abst 8:894, 1982), implicating individual differences in responsivity to striatal dopamine (DA) stimulation in variability in these behaviors.

To examine the relation between responsivity to striatal DA stimulation and ESLH, rats (n=24) with bilateral striatal cannulae and LH electrodes were tested for behavioral response to the DA agonist (-)N-n-propylnorapomorphine HCl (NPA) in striatum and to ESLH. First, durations of oral behavior (gnaw, lick, eat, groom) were recorded for 75 min after 2 unilateral striatal infusions of 10 μg NPA/1 μl/5 min, and after an intervening vehicle infusion, at progressive depths through each guide cannula. Next, rats were screened on 2 occasions for duration of consumption (eat, drink, gnaw) induced by ESLH through each electrode at the lowest current which evoked consumption on 9/10 consecutive 20 sec trials. Later, a deeper NPA infusion was administered, to ensure maximal DA stimulation yet minimize effect of striatal damage on ESLH-tests.

While variability in duration of oral behavior evoked by NPA (ave. minus vehicle response) was great, ranging from 0-58% of total time, behavior evoked through the 2 cannulae within each rat was highly correlated (r=.84, $p<.001$). Duration of ESLH-evoked consumption (range=0-85% total time) for each electrode was significantly correlated with NPA-induced oral behavior through the corresponding ipsilateral cannula across rats (r=.60, $p<.001$). However, several rats responded well bilaterally to NPA and only unilaterally to ESLH: asymmetry in NPA response was unrelated to asymmetry in ESLH response (r=.29). Across rats, best ESLH response between the 2 electrodes was very well correlated with either best NPA response between the 2 cannulae (r=.82, $p<.001$) or with average NPA response between the 2 cannulae (r=.79, $p<.001$). Histology confirmed that neither cannula nor electrode position determined behavioral variability. It appears then that oral behavioral responsivity to striatal NPA is related to magnitude, though not to asymmetry, of ESLH-evoked consumption among rats.

We conclude that individual rats respond similarly in degree of external stimulus-directed behavior elicited by striatal DA stimulation or ESLH. These results are also consistent with evidence that stimulation of ascending DA fibers contributes to ESLH effect.

286.16 UP-DOWN REGULATION OF DOPAMINE RECEPTORS INDUCED BY DIFFERENT AFFECTIVE COMPONENTS OF A STRESS SITUATION. Graham J. Bean and Tyrone Lee. Psychopharmacology Unit, Clarke Institute of Psychiatry, Toronto, Ontario, CANADA M5T 1R8.

Exposure to unavoidable shock stress in the rat has been reported to be associated with an increase in dopamine receptors in the frontal cortex, and a decrease in the striatum, hippocampus and diencephalon (Cherek et al., Soc. Neurosci. Abstr., Vol. 6. p. 543, 1980). We now report that different affective components of a stress situation may selectively up or down regulate dopamine receptors in the rat.

Weight-matched pairs of male Long-Evans hooded rats (175-225gm) were exposed to 4 daily 2-hour sessions of electric shock stress administered on a fixed interval (22 sec) schedule of reinforcement. Subjects were placed in individual plexiglas chambers mounted on top of a 2 ft diameter running wheel. For the avoiding animal, turning the wheel ½ revolution during a 6 sec tone terminated the tone and the possibility of impending tail-shock (3.8 mA; 1 sec). The yoked animal received the same distribution and duration of tone and shock stress as the avoiding animal but was unable to control the offset of shock. The control group was exposed to identical experimental procedures except that the source of shock was turned off. On completion of the last test session, rats were decapitated and subsequent striatal homogenates were prepared for dopamine receptor (D_2) assay using ^{3}H-spiperone and 10 μM sulpiride to define specific binding. Scatchard analyses were performed to determine the density (B_{max}) and affinity (K_D) of dopamine receptors.

On the basis of avoidance performance on day 3 and on day 4, the following observation was made: When the avoiding animal made consistent shock avoidance (> 65±11%) on day 3 and on day 4, the resulting B_{max} was not significantly different from control or yoked animals. When avoiding animals made a dramatic reduction in avoidance (> 54%) from day 3 to day 4, there was a significant decrease in B_{max} from control values. When avoiding animals made a substantial improvement in shock avoidance from day 3 to day 4, typically terminating the last session with 75% avoidance or better, the resulting B_{max} was not significantly different from controls. In contrast, the resulting B_{max} for the corresponding yoked animals was highly elevated. The receptor affinities (K_D) were not significantly different between groups.

Our present behavioral data tend to support the view that under a stress situation when animals lose control, there is a corresponding decrease in the density of brain dopamine receptors. However, under conditions of 'chronic expectancy' (as in the case of yoked animals receiving relatively little shock on day 4 as opposed to day 3), there is a significant increase in the density of these receptors. (Supported by the Clarke Institute of Psychiatry).

287.1 CHANGES IN THE GEOMETRY OF RABBIT CILIARY GANGLION CELLS AFTER THE INTERRUPTION OF PRE- OR POSTGANGLIONIC AXONS, D.A. Johnson, Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, Missouri 63110.

Adult rabbits were anesthetized and the ciliary ganglia were subjected to either postganglionic nerve section or, in other experiments, intraorbital section of the preganglionic fibers in the oculomotor nerve. Ganglia were removed 3-28 days later and studied in vitro. The innervation of individual neurons was characterized electrophysiologically; ganglion cells were then injected with horseradish peroxidase (HRP) and examined in whole mounts after development of the HRP reaction product.

Following postganglionic axotomy, the average dendritic length increased and growth cone-like structures appeared along the terminal branches of many dendrites. The number of processes arising from the cell body, however, remained approximately the same as in control neurons. In contrast, after preganglionic nerve section there was an increase in the number of processes arising from ganglion cell bodies. Many of these processes bore varicosities similar to synaptic boutons. In accord with this anatomical observation, in some animals antidromic stimulation of the postganglionic (ciliary) nerve elicited synaptic potentials in ganglion cells as early as 4 days after denervation. Since intraganglionic connections are normally either absent or present only in small numbers (Johnson and Purves, 1981), these sprouted processes evidently make new synaptic contacts.

These results show that interruption of either peripheral projections or preganglionic inputs leads to marked changes in ganglion cell geometry within a few days. Loss of contact with the periphery evidently induces growth of pre-existing dendrites, whereas interruption of preganglionic innervation initiates the extension of new processes from the cell body, some of which probably form novel synaptic connections. These findings suggest that the adult geometry of these neurons arises from a balance of influences deriving both from the periphery and from the normal distribution of preganglionic innervation.

Reference

Johnson, D.A. and Purves, D., J. Physiol. 318: 143-160.

This work was supported by USPHS Grant No. NS 11699 and 18629 to D. Purves.

287.2 EARLY DEGENERATIVE AND REGENERATIVE RESPONSES IN TRANSECTED SPINAL AXONS OF THE LAMPREY. M. K. McHale* and M. J. Cohen. Department of Biology, Yale University, New Haven, CT 06511. (SPON: M. J. O'Donovan).

Spinal cord transection of the larval lamprey, Petromyzon marinus, results in sprouting of the giant reticulospinal neurons and the establishment of new synaptic contacts distal to the lesion by 100 days. The giant axons initially react to axotomy by retrograde retraction or "die-back" for several hundred micrometers.

To refine observations on die-back and early regeneration, we serially sectioned the 1 mm segment of spinal cord proximal to the lesion in animals 5 and 7 days after transection. Light and electron microscopic studies revealed that, at 5 days, the endings of 19 giant axons were at an average of 280 um proximal to the lesion site. Two morphologically distinct endings could be identified among these axons; 5 fibers ended in a swollen bulb; the remaining 14 endings showed a gradual tapering.

In both types of endings, accumulations of vacuoles, vesicles, mitochondria, and microtubules were localized to the most distal portion. In the proximal areas of the bulb, these accumulations were confined to the periphery while the central areas contained axoplasm in a swirled configuration and organelles in various planes of alignment. In the proximal areas of tapered endings, this configuration was absent; however, at an average distance of 100 um from the tapered tip, a dense core of twisted neurofilaments, averaging 100 um in length, occupied the center of the axon.

Axons were first seen to sprout 7 days after axotomy. Eight spinally-transected animals were examined; sprouting was seen in 6 out of 8 animals. Ten out of 11 sprouts emerged, usually at right angles, from the membrane of the terminal bulb while the remaining sprout projected from the tip of the axon. In the former, the position of emergence was, on the average, 330 um proximal to the lesion and 45 um proximal to the tip of the parent axon. The mean diameter of the sprouts was 6 um and the mean length 30 um. Sprouts most commonly coursed in a distal direction, parallel to the parent fiber.

The emerging sprouts demonstrated distinct differences in the proportion and localization of cytoplasmic elements. The base of the sprouts resemble parent axoplasm but contain more vesicles, with the highest concentration of vesicles (clear, dense-core and coated) at the leading edge of the sprout. The tip of the sprout also contains microtubules and vesiculo-tubular profiles in a granular matrix. Neurofilaments are notably absent.

Giant axon morphology of 5 and 7 day spinally-transected larval lampreys shows that die-back precedes regenerative responses and that sprouting occurs early, at some distance proximal to the lesion. Outgrowths usually emerge at the bulbous endings of the severed axons, most commonly at right angles, and then turn either caudally or rostrally.

(Supported by NIH Spinal Trauma Grant NS 10174).

287.3 AXOTOMY INDUCES BOTH DENDRITIC SPROUTING AND RETRACTION IN LAMPREY CENTRAL NEURONS. G. F. Hall* and M. J. Cohen. Biology Department, Yale University, New Haven, CT. (SPON: K. Herrup).

The effect of axotomy on the morphology of anterior bulbar cells (ABC) in the larval sea lamprey has been recently found to depend upon the distance of the axonal lesion from the soma (Hall & Cohen, submitted to Science, 1983). "Close" axotomy within 500 um of the soma evoked extensive dendritic sprouting beyond the limits of the normal dendritic tree in these cells without significant axonal regeneration. Sprouts were readily identifiable by their swollen tips and relative lack of branching. Axotomy at sites 1 cm or more from the soma resulted only in axonal sprouting.

We report here that a marked retraction of axotomized ABC dendrites was seen after long periods post axotomy. Cells were examined in whole mount after intracellular injection of Lucifer Yellow. Dendritic retraction was assessed visually and was characterized by a narrowing of the primary and secondary dendritic trunks and an outright loss of finer branches. The soma was usually swollen, while resting membrane and action potential amplitudes remained normal.

This phenomenon compares with dendritic sprouting following axotomy as follows: 1) Dendritic retraction was usually seen several months post axotomy, while dendritic sprouting begins within 2-3 weeks. 2) Dendritic retraction is not dependent on the site of axotomy, and was found in all axotomized cells examined after 200 days post axotomy regardless of lesion site, while dendritic sprouting is strongly site dependent. 3) Both phenomena were found to be caused only by axotomy; cell damage and deafferentation caused by amputation of ABC lateral dendrites without axotomy evoked neither sprouting from nor retraction of the dendrites.

Dendritic retraction started to appear in some cells between 50 and 80 days following "close" axotomy. These same cells also exhibited extensive dendritic sprouting beyond the normal dendritic limit despite the retraction of dendrites within the confines of the normal dendritic tree. By 250 days after "close" axotomy, all cells examined showed severe dendritic retraction. Few axotomy-induced dendritic sprouts were present, suggesting that these sprouts had retracted considerably by this time.

We suggest that dendritic sprouting and dendritic retraction may act via separate mechanisms, each set in motion by axotomy. It has been suggested (Sumner and Watson, Nature, 1971) that dendritic retraction can result after disconnection from a postsynaptic target, which might explain the lack of dependence of dendritic retraction on the site of axotomy in ABC's. On the other hand, the strong dependence of dendritic sprouting on the location of the axon lesion suggests that the axonal injury itself is the factor controlling dendritic sprouting. (Supported by NIH Spinal Trauma Grant NS 10174).

287.4 PROTEIN-PRECURSOR INCORPORATION BY THE PRESUMPTIVE CELLS-OF-ORIGIN OF SPROUTING INPUTS TO THE DENTATE GYRUS. B. Fass and O. Steward. Neurosurg. Dept, Univ. Virginia Sch. Med., Charlottesville, VA

Metabolic alterations occur within the denervated neuropil of the dentate gyrus (DG) during the period of reinnervation which follows a lesion of the entorhinal cortex (EC); they include increases in 2-deoxyglucose uptake and protein-precursor incorporation (Steward & Smith, 1980; Fass & Steward, 1983). The present study focused on whether there are metabolic changes within the presynaptic cells which participate in the reinnervation; namely, the cells-of-origin of the crossed temporodentate (CTD) pathway.

Adult male albino rats sustained a unilateral EC lesion and survived for periods from 2 to 60 days postlesion (DPL). Thirty min prior to sacrifice, each rat was injected intravenously (via the tail) with 3H-leucine (2.5uCi/gm body-weight). The rats were perfused with formalin and their brains were prepared for quantitative autoradiography. Silver grains (reflecting the incorporation of 3H-leucine into protein) were counted over stellate cells in layer II of the dorsal EC contralateral to the lesion. Such cells in the most medial portion of the EC participate in reinnervating the DG, whereas counterparts in the intermediate portion evidently do not (Steward & Vinsant, 1978). Thus, we computed ratios of the density of grains over stellate cells in the most medial region and in the intermediate region of the contralateral EC; the ratios then were normalized by expressing them as a percent of the corresponding value for intact-control cases.

Beginning at 4 DPL, there was an increase in grain density per cross-sectional area of cell body and a larger increase in grain number per cell body. Grain density returned to control levels by 10 DPL. Grain number per cell body peaked between 4 and 8 DPL, remained above control levels between 10 and 20 days, and returned to control levels by 60 days.

The time of the largest increase in leucine incorporation by stellate cells in the most medial portion of the contralateral EC corresponds almost exactly to the time when the number of intact axon terminals in the denervated zone of the DG begins to return toward control values; thus, there is a 3-fold increase in terminal density in the denervated zone during the time that the cells-of-origin of sprouting fibers exhibit an increased incorporation. At later postlesion intervals when the new terminals apparently begin forming mature synapses within the denervated zone (10-14 DPL), the increase in leucine incorporation is not as great. Since these stellate cells presumably are the ones giving rise to new axon terminals of the CTD pathway, the present finding suggests that lesion-induced growth of these terminals may be preceded by an alteration in protein metabolism within the soma of the cells-of-origin.

Supported by NIH grant NS12333 and RCDA NS00325 to O.S.

287.5 THE ROLE OF SENSORY AND MOTOR NEURONS IN THE TRANSNEURONAL INDUCTION OF SPROUTING, G. Ring*, H. Sugerman* and S. Rotshenker. Hebrew University-Hadassah Medical School, Jerusalem, Israel.

Previous studies (e.g. Rotshenker, S., J. Neurosci., 2, 1358-1361, 1982) indicate that axotomy of one cutaneous-pectoris (c.p.) nerve initiates a signal for growth in the injured neurons somata that is transferred transneuronally to the intact c.p. motor neurons on the opposite side. Consequently, the intact nerve sprouts, forms additional synapses, and a pattern of polyneuronal innervation (P.I.) develops (37.4±3.3% v.s. 17.6±1.1%). The c.p. nerve contains both motor and sensory nerve fibers. We were therefore interested to examine the relative role of motor and sensory neurons in the transneuronal induction of sprouting. To examine the role of injured motor neurons in the transneuronal induction of sprouting, right muscles were examined electrophysiologically after either combined lesions to left 2nd dorsal roots (D.R.) and left c.p. nerves or to left 2nd ventral roots (V.R.). An increased pattern of P.I. in right muscles was observed after combined left D.R. and c.p. nerves cuts (36.6±2.3% v.s. 17.6±1.1%) but not after severing V.R. only. To examine the role of injured sensory neurons, right intact c.p. muscles were examined either after cutting left 2nd D.R. or after a combined lesion to left c.p. nerves and left 2nd V.R. In neither instance was there any change from normal. To examine the role of intact sensory neurons right muscles were examined after combined lesions to left c.p. nerves and right D.R. No altered pattern of innervation developed. It is thus concluded: 1) injured motor neurons play a major role in the transneuronal induction of sprouting. 2) a lesion placed in too close proximity to the motor neurons cell body does not induce transneuronal sprouting. 3) deafferentation interferes with the motor neurons ability to respond with sprouting to contralateral axotomy.

287.6 MOTOR NEURON SPROUTING FOLLOWING CONTRALATERAL AXOTOMY IN MAMMALIAN MUSCLES. M. Tal and S. Rotshenker. Hebrew University-Hadassah Medical School, Jerusalem, Israel.

We have previously demonstrated that intact nerves that innervate skeletal muscles in the frog sprout and form new synapses after injuring homologous motor nerves on the contralateral side (e.g. Rotshenker, S., J. Neurosci., 2, 1359-1368, 1982). The present study was undertaken to examine whether or not a similar phenomenology also occurs in the mammalian motor system. To do so we examined anatomically intact mice peroneus tertius muscles following axotomy of the sciatic nerve that contains motor axons that innervate the contralateral muscles. Muscles were sectioned 30 microns thick and stained with the combined silver cholinesterase stain that visualizes motor axons, nerve endings and end-plate regions. On the average, about 100 synapses were examined in each muscle under a total magnification of 400, and the incidence of sprouting defined as the percentage of synapses that were innervated by both a myelinated axon and a sprout. The incidence of sprouting in intact muscles of unoperated mice was 5.1±2.2% (SEM, 4 muscles examined). This incidence increased about 4 fold 1 to 7 weeks after contralateral axotomy and reached an average of 22.7±1.4% (SEM, 11 muscles examined). Thus in mice, as in frogs, axotomy of a motor nerve may be followed by the sprouting of intact motor neurons situated contralateral to the side of the axotomy. There is, however, a difference in the sprouting response between mammals and amphibians. In mice sprouts contributed to the innervation of muscle fibers already innervated by the parent axon. In the frog, sprouts commonly hyper innervated muscle fibers already innervated but not by the parent axon.

287.7 ABSENCE OF SYMPATHETIC SPROUTING IN THE RAT OLFACTORY BULB FOLLOWING CHOLINERGIC DENERVATION. K.A. Crutcher and W. A. Gibby. Department of Anatomy, University of Utah School of Medicine, Salt Lake City, Utah 84132.

The growth of sympathetic noradrenergic fibers into cholinergic-denervated hippocampal and neocortical regions has led to the suggestion that such sprouting may be a general response within the CNS. A test of this hypothesis was conducted by denervating the rat olfactory bulb of cholinergic input and examining the histofluorescent appearance of the bulb at various survival times. Electrolytic lesions were placed within the horizontal limb of the diagonal band and in some cases the superior cervical ganglion (SCG) was autologously transplanted to the region of the bulb. Sections stained for acetylcholinesterase activity revealed a profound depletion of the bulb ipsilateral to the lesion by five days. In normal animals the bulb contained central noradrenergic fibers but sympathetic fibers were only present in small numbers adjacent to pial blood vessels. This appearance was unchanged even as long as four months following cholinergic denervation of the bulb. In the cases where the SCG transplants survived, the transplant contained numerous perikarya which exhibited noradrenergic histofluorescence and acetylcholinesterase activity. There was also a dense plexus of fluorescent fibers within the transplant and some fibers were found along the blood vessels and meninges. However, there was no evidence of innervation of the bulb by the SCG perikarya even as long as four months after the surgery. Since the cholinergic neurons projecting to the neocortex reside in the same basal forebrain region as those innervating the bulb, injections of fluorescent markers (primuline and bisbenzimide) were made in order to determine whether the same cells projected to both places. The results indicate that the bulb and neocortex receive cholinergic input from completely separate populations of neurons. These observations do not support the hypothesis that sympathetic sprouting in response to cholinergic denervation is a general response within the CNS. What conditions permit this response in some CNS regions and not in others remain to be determined. The demonstration of a brain region where cholinergic denervation does not elicit sympathetic ingrowth may facilitate elucidation of the mechanism in regions where it does.

(Supported by NSF Grant BNS 81-03678.)

287.8 HYPOTHYROIDISM PREVENTS LESION-INDUCED NORADRENERGIC SPROUTING. Zehava Gottesfeld and Ian Butler*. Dep. of Neurobiology and Anatomy and Dep. of Neurology, Univ. Texas Med. Sch. Houston, TX 77025.

This study examined the effect of propylthiouracil (PTU), an antithyroid drug, on lesion-induced sprouting of intact noradrenergic (NA) axon terminals in the partially deafferented habenula (Hb). This drug was administered to pregnant rats in their drinking water (50 mg/lit) beginning from the 12th day of pregnancy and terminated when the pups were weaned at 25 days of age. These dams received, in addition, daily injections of thyroxine (25 ug/kg, s.c.). After weaning the pups were separated from their mothers and only male pups were then used in this study. These pups were divided into 2 groups (20 rats each), one group (control), received food and water ad lib and the second group (PTU), had free access to food, except that the drug (50 mg/lit) was added to their drinking water. At 6 weeks of age part of the rats in each group received stria medullaris lesions (SMX), using high frequency current; this lesion partially deafferents the Hb. The PTU rats continued to receive the drug for 4 weeks post-lesions, and the last treatment was given one day prior to being sacrificed. Sprouting of NA axon terminals in the Hb was identified chemically, by changes of norepinephrine (NE) levels and morphologically, by histofluorescence using the glyoxylic acid method. Habenular NE levels (ng/mg prot.) in the 4 groups were: (1) control/no lesion: 13.1+1.0 (2) control/SMX: 22.2+2.2 (+71% vs. group 1); (3) PTU/no lesion: 17.4+1.4 and (4) PTU/SMX: 12.9+0.8. The histochemical demonstration revealed increased intensity of fluorescence associated with marked proliferation of axon terminals in the control/SMX group. However, the PTU/SMX group was similar to control/no lesion group. The major finding in this work is that lesion-induced NA sprouting is suppressed by PTU-induced hypothyroidism.

Supported in Biomedical Research support grant.

287.9 COMPETITION REGULATES GROWTH OF 5-HT AND NE AXONS IN IMMATURE CEREBRAL CORTEX. M.E. Blue, M.E. Molliver, M.S. Lewis*, E.M. Glaser and H.G.W. Lidov. Dept. of Cell Biology & Anatomy, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205, Dept. of Physiol. Univ. of Md.. Sch. of Med., Baltimore, MD 21201.

The early development of noradrenergic (NE) neurons and their widespread projection to all cortical areas has led to the proposition that these neurons have a regulatory role in brain development. A recent investigation (Blue and Parnavelas, 1982) has provided evidence indicating that the NE innervation exerts a powerful influence upon synaptogenesis in the visual cortex of rats in the immediate postnatal period. In that study, following the removal of the NE afferents by systemic injections of 6-hydroxydopamine (6-OHDA), a striking increase was observed in the total number of synapses in the visual cortex during the first postnatal week. These results suggest that following destruction of the NE innervation, there is sprouting by other unidentified axons. Based on that data we have postulated that the raphe-cortical serotonergic (5-HT) axons undergo sprouting in the cortex of 6-OHDA treated rats and contribute to the increased density of synapses. To test that hypothesis, experimental animals were administered 6-OHDA (one to four doses 100 µg/gm s.c.) in the first four days of postnatal life; vehicle injected littermates served as controls. At postnatal day 6 the 5-HT innervation of cerebral cortex was visualized in frozen sections by immunocytochemistry employing an antiserum to 5-HT (using peroxidase-anti-peroxidase or avidin-biotin-peroxidase complex methods). The density and the distribution of 5-HT axons in selected cortical areas was assessed by light microscopy, and the length of 5-HT axons within a measured area of the section was determined quantitatively and graphically using the Glaser-Van der Loos Image Combining Computer Microscope.

Preliminary qualitative and quantitative observations demonstrate an increased density of 5-HT axons in the cerebral cortex of the 6-OHDA treated rats. The augmentation of 5-HT axons is most evident in cortical layers I-IV. The laminar distribution of this 5-HT sprouting is coextensive with the increased density of synapses previously reported in 6-OHDA treated rats. In addition, the total length of 5-HT-positive axons is significantly increased (ca. 20-30%) in somatosensory and visual cortex. These results provide evidence that there are trophic interactions between cortical afferents in development, specifically, there is competition between NE and 5-HT axons as evidenced by sprouting and synaptic reorganization which follows ablation of the coeruleo-cortical axons. (Supported by NIH grants NS-15199, RR5378, MH-15330 and UCP R-340-83)

287.10 COLLATERAL SPROUTING OF NORADRENERGIC FIBERS INTO RAT BRAINSTEM AND CEREBELLUM AFTER DORSAL BUNDLE LESIONS IN NEONATAL RATS; FAILURE OF 6-HYDROXYDOPA TO MODIFY THE RESPONSE. Richard M. Kostrzewa and Dolores Klisans-Fuenmayor. East Tenn. State Univ., Quillen-Dishner College of Med., Johnson City, TN 37614.

Sprouting of noradrenergic fibers into the cerebellum after mechanical lesions of collateral tracts has been demonstrated in adult animals by several groups. In order to demonstrate whether the responsiveness of this process would differ in developing animals, knife cuts of the dorsal noradrenergic bundle were made at the level of colliculi, in rats at birth, 3 days postbirth or 5 days postbirth. Animals were sacrificed at 6 weeks, and brains were removed for (1) norepinephrine (NE) assay by a trihydroxyindole fluorometric method and (2) glyoxylic acid histofluorescence microscopy, for visualization of the noradrenergic fiber network. As demonstrated by elevations in NE content and/or by increased density of fluoroescent fiber networks there was an ensuing noradrenergic hyperinnervation of both the pons-medulla and cerebellum, when lesions were made at birth, 3d or 5d postbirth. When the knife cut was made at a more rostral position, at the level of the posterior hypothalamus, noradrenergic hyperinnervation of the cerebellum and pons-medulla did not occur. When the lesion was made at a position slightly caudal from the mid-collicular cut, noradrenergic sprouting again was not observed. Given in conjunction with each different lesion, a low dose of the neurotoxin 6-hydroxydopa (6-OHDOPA) (20 µg/g) failed to modify the response. These findings indicate that a suitable lesion in neonatal brain will result in the collateral sprouting of noradrenergic neurons; concurrent damage of the sprouting network by 6-OHDOPA does not alter the capability of the system. Since the degree of noradrenergic sprouting is comparable to that observed in older rats, it becomes apparent that plasticity of this system remains intact and little changed even after development. (Supported by NS 14797.)

287.11 AXONAL SPROUTING INVOLVES NERVE GROWTH FACTOR. C.E. Hulsebosch, J.R. Perez-Polo+ and R.E. Coggeshall. Marine Biomedical Institute, +Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, Texas.

Dorsal root axons are reported to sprout following either spinal hemisection or unilateral dorsal rhizotomies above and below a spared root. To provide axonal numbers, we counted dorsal root fibers in the electron microscope, which can resolve both myelinated and unmyelinated axons. We found a 10-15% increase in the number of unmyelinated axons in the roots on the operated side of rats when either surgery was done within the first month of life (Science, 213: 1020-1021; Brain Research, 224: 170-174). The role of NGF in regeneration of sensory neurons, a peripheral target of NGF in development, is not well understood. Similarly, little is known about the role, if any, of dorsal root ganglia in hemisection paradigms designed to study axonal sprouting.

The present study was designed to test the involvement of endogenous (NGF) on the sprouting response of dorsal root axons. Hemisections were done in rats at birth and daily subcutaneous injections of the antibody to NGF were given in doses (3 µl whole rabbit sera/gm. weight) sufficient to bind and render endogenous levels of NGF inactive. Shown below are unmyelinated axonal counts from four pairs of dorsal roots of the first cranial segment above the hemisection.

	RAT 1	RAT 2	RAT 3	RAT 4
HEMISECTED SIDE	3935	4462	3829	4641
CONTROL SIDL	5297	4690	4920	3954

These figures indicate a decrease in the number of unmyelinated axons in dorsal roots of the hemisected as compared to the normal side. This decrease is statistically significant ($p<.05$). Since untreated hemisected rats demonstrate an increase in unmyelinated axons which we interpret as sprouting, the decrease in the unmyelinated axon population of hemisected rats treated with anti-NGF may be interpreted as inhibition of the sprouting response. The situation is more complex, however, for there is now a surplus of fine fibers on the normal side. Whatever the precise mechanism, the data seems to indicate that endogenous NGF is involved in the sprouting of dorsal root axons after spinal cord denervation. These results are consistent with the hypothesis that interuption of the availability of NGF to dorsal root axons by antibody could be interpreted by neurons whose axons are not cut as a signal to sprout. Supported by BRSG SO7-RR05427 and SO7-RR07205 (C.E.H.), NS18707 and Moody Foundation (J.R.P.-P.) and NIH grants, NS10161, NS17039, NSO7377, NS11255 and Moody Foundation (R.E.C.).

287.12 TREATMENT WITH GANGLIOSIDES OF THE ALTERATIONS IN NEURONAL CONDUCTION VELOCITY, REGENERATION AND TRANSPORT IN DIABETIC NEUROPATHY, A. Gorio, F. Di Gregorio*, R. Canella*, A. Schiavinato*, R. Siliprandi*, F. Norido*, R. Zanoni* and M. Vitadello. Dept. of Cytopharmacology, Fidia Research Laboratories, Abano Terme (PD), Italy.

We have investigated the development of neuropathy in genetic diabetic mice (db/db) and alloxan treated rats. The conduction velocity (NCV) of db/db mice is lower throughout the life of the animal compaired to the heterozigote.

The decrease in conduction velocity is accompanied by a reduced axonal diameter particularly significant at 180 days of life. At this age these changes are accompanied by a reduced axonal flow of AChE light forms, while previously no changes were ever observed. Treatment with insulin improves nerve conduction velocity and axonal morphometry only prior to the changes in AChE transport. At this latter stage ganglioside treatment becomes effective improving NCV and axonal morphometry. Thus we have two phases of diabetic neuropathy a "metabolic" one sensitive to insulin and a "neuronal" one sensitive to ganglioside. Injections of ^{35}S-methionine in the ventral horn allowed to evaluate the transport of cytoskeletal proteins. The shape of radioactivity distributions along the nerve showed no evident peak and made it very hard to establish unambiguously the transport velocity. To circumvent this difficulty we used the front of the wave to get a measure of maximal velocity (Vmax) and the average distance travelled by the wave to estimate the average velocity (Vav) (the average distance is here defined as $(\Sigma r\ d)/\Sigma r$, where r is the radioactivity of a given 3 mm nerve segment and d its distance from the injection site).

TRANSPORT VELOCITIES (mm/day)

	Control		Diabetic	
Protein	Vmax	Vav	Vmax	Vav
Actin	3.1	0.76	3.1	0.66
Tubulin	1.2	0.95	1.2	0.64
NF145	1.0	0.54	0.8	0.40

Transport of these cytoskeletal proteins is overall retarded. Actin and tubulin in diabetic nerves seem to be hold back for a longer time. On the contrary, NF 145 shows a front which advances at a lower speed and does not trail more than the control, as if this protein was, as a whole, being conveyed more slowly in diabetic mice. Studying EDL reinnervation in alloxan treated rats we found that the rate of axonal regeneration (elongation) was effected by a 40% reduction; sprouting polyinnervation and synaptic repression were delayed, but otherwise unaffected. These results would suggest that impairment of axonal transport may affect axonal elongation rather than sprouting.

287.13 INDUCED HOMOTYPIC SPROUTING OF HIPPOCAMPAL 5-HT FIBERS: AN IMMUNOCYTOCHEMICAL STUDY IN THE RAT. F.C.Zhou* and E.C.Azmitia. (SPON: J.E.Shriver). Department of Anatomy, Mount Sinai School of Medicine, New York, NY 10029.

The dorsal hippocampus (DHipp) of the rat normally receives 5-HT innervation from two homologous groups of cells in the median raphe nucleus via the cingulum bundle (CB) and the fornix-fimbria (FF) (Zhou and Azmitia, Brain Res.Bull.,10:4,1983; Azmitia and Segal, J.Comp . Neurol.,179:641,1978). Selective lesioning of the 5-HT fibers in the CB induces axonal and cellular redistribution of these two groups of neurons and results in functional abnormality. Structural, biochemical and functional restoration has been observed within this model system of the long-term CB-lesioned animals (Azmitia et al, Nature, 274:374,1978; Zhou and Azmitia, Soc Neurosci Abstr.,7:68, 1982; Clewans and Azmitia, Soc Neurosci Abstr.,7:744;1982). We present here direct anatomical evidence that restoration from the CB-lesion is a result of homotypic reinnervation of 5-HT fibers in the DHipp through the undamaged FF.

Animals were unilaterally microinjected with 5,7-DHT (4 ug in 400nl) into the CB and sacrified 3(n=3), 14(n=3) and 42(n=3) days post-lesion, along with sham-lesioned (n=2) and normal animals (n=3). The animals were pretreated with pargyline and L-tryptophan (200mg/kg) prior to perfusion with 4% paraformaldehyde in phosphate buffer. Brains were sectioned through the hippocampus and the 5-HT immunoreactive fibers stained with 1:2000 anti-5-HT antiserum (from J.Lauder).

In normal and sham rats, 5-HT immunoreactive (5-HT-IR) axons free of varicosities were observed in the medial CB and the FF. Fine distorted 5-HT-IR fibers with large varicosities were densely distributed in the stratum lacunosum-moleculare and stratum oriens of cornu Ammonis and in the infra-granular layer of the dentate gyrus, especially in the fasiola cinereum (FC), and sparsely distributed in the rest of the DHipp. These fibers were diminshed in the CB and decreased in density in the DHipp, especially in CA1 and FC at 3-day CB-lesion. Dark droplet-like stumps (DDS) were spotted. No appreciable changes in density or pattern of 5-HT-IR fibers were observed at 14-day as compared to 3-day post CB-lesion but there were less DDS.

However, at 42-day after CB-lesion, the 5-HT-IR fibers had greatly increased and were as densely distributed as in the normal DHipp. The fibers were denser in the contralateral FC compared to the ipsilateral FC. No 5-HT-IR fibers were seen in the ipsilateral CB, but a dramatic increase of 5-HT-IR fibers in the ipsilateral fimbria was observed as compared to the contralateral side. NSF-grant BNS-79-06474.

287.14 NORADRENERGIC HYPERINNERVATION ALTERS RESTING MEMBRANE PROPERTIES AND RESPONSE TO SYNAPTIC INPUT. James J. Vornov* and Jerome Sutin Department of Anatomy, Emory University School of Medicine, Atlanta, Ga. 30322

The noradrenergic hyperinnervation of the rat motor trigeminal nucleus produced by the neonatal administration of the neurotoxin, 6-hydroxydopamine (6-OHDA) was used as a model to test the hypothesis that developmentally increasing the density of an existing CNS projection increases its physiological effect. In both control and neonatal 6-OHDA treated rats, the noradrenergic innervation arises from cells in the region of the lateral lemniscus (Vornov and Sutin, J. Comp. Neur. 214: 198-208, 1983). We have previously found (Vornov and Sutin, Anat Rec 205: 207A, 1983) that stimulation of the LL region produces a strong facilitation of the masseteric reflex which is reduced by systemically administered alpha- and beta-adrenergic receptor antagonists. The mean peak facilitation is 71% larger in the neonatal 6-OHDA treated animals than in controls, consistent with the hypothesis that the increased density of the projection produces an increased physiological effect.

The mean resting potential of hyperinnervated motor neurons was -60.34 ± .75 mv, which was 3 mv more hyperpolarized than controls (-57.35 ± .54 mv). The input resistance was reduced by 33% from 1.83 ± .15 megohms in normal cells to 1.22 ± .19 megohms. The peak amplitude of the MesV-evoked EPSP in hyperinnervated motorneurons was increased by 1.3 mv (+71%) and the half width increased by 0.7 msec (+26%). Stimulation of the LL region produced a predominantly depolarizing PSP which was correlated with the period of reflex facilitation. The peak amplitude of the depolarization in hyperinnervated motorneurons was not significantly different from controls. Input resistance decreased, but stimulation of MesV during the PSP produced an EPSP of increased amplitude. The reflex facilitation produced by LL region stimulation, then, arises from both a depolarizing PSP and from an increase in the MesV-evoked EPSP's amplitude. These results indicate that a major synaptic action of norepinephrine was facilitation of non-noradrenergic synapses. The response to stimulation of the increased noradrenergic projection was greatly enhanced and resting properties and response to synaptic input were altered. The effect of hyperinnervation on MesV-evoked EPSP amplitude and on input resistance was similar to the effect of LL region stimulation, indicating that the increased density of noradrenergic terminals is tonically active.

This work was supported by grant NS-14778, NIH Medical Scientist Training Program grant GM-07415, and the Hellen Miller Endowment award to Emory University.

SPROUTING II

288.1 GROWTH OF AXONS IN THE SOMATOSENSORY THALAMUS FOLLOWING LESIONS OF THE DORSAL COLUMN NUCLEI. J. Wells and T. H. Melander*. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405

Following lesions of the dorsal column nuclei (DCN), the number of synapses in the ventral posterolateral nucleus (VPL) of the thalamus first decreased and then returned to normal (Tripp and Wells, Brain Res. 115:362, 1978). A likely source of these new terminals was a population of neurons that were undamaged by the lesion and were located caudal to the DCN (Daniloff, Wells and Tripp, Anat. Rec. 202:41A, 1982). In the present experiments the antegrade transport of HRP conjugated to wheatgerm agglutinin (WGA/HRP) labeled the axons in VPL which originated in or passed through the injection site. Large bilateral injections of WGA/HRP were made into the region caudal to DCN lesions one day before the brain was processed for the TMB reaction. Two groups of rats were studied. A short term group received the lesion 6-8 days before the brains were analyzed and a long term group was analyzed 50-85 days after the lesions. The DCN lesions were unilateral.

All the lesioned brains contained fewer axons in VPL than normal. The animals in the short term group showed two patterns of distribution of axons in VPL. 1.) Labeled axons occupied only a band of fibers along the lateral margin of VPL and the rostral and caudal zones that are transitional between VPL and adjacent nuclei. In this pattern there were no labeled axons in other parts of VPL. 2.) Less frequently, fibers were seen throughout VPL. The labeled axons were scattered individually in VPL but rarely reached the medial margin of the nucleus.

In the long term group, the distribution of labeled axons varied markedly. In some animals a prolific growth of axons resulted in a pattern which approached that of a normal distribution. Other animals showed a moderate growth of axons yielding a pattern of individual fibers distributed throughout VPL. Unlike the short term group areas within VPL and particularly along its medial margin showed dense accumulations of reaction product which suggested that these patches were sites of intensive growth. In a few rats no reaction product was seen in VPL and only a minimal amount was observed in the transition zones.

The results indicate that under some conditions considerable growth of axons is possible in the adult VPL. The axonal growth seems to spread out from patches of intensely labeled axons that have survived the DCN lesions. These studies also suggest that the restoration of the normal number of synapses can be achieved without a normal complement of axons in VPL.
Supported by PHS 5429-17-8

288.2 ELECTROPHYSIOLOGICAL CORRELATES OF SPROUTING FOLLOWING UNILATERAL ENTORHINAL CORTEX LESIONS. T.M. Reeves, D.C. Smith, & R.A. Jensen. Developmental Biopsychology Lab, Southern Illinois University, Carbondale, IL 62901

After unilateral lesions of the entorhinal cortex in adult rats, some remaining afferents to the dentate gyrus increase their terminal field through collateral sprouting. One dentate input that shows such postlesion growth originates in the unlesioned entorhinal cortex and terminates in the outer 2/3 of the contralateral dentate molecular layer (Steward, Cotman & Lynch, 1976, Brain Res., 114, 181-200). In addition to autoradiographic evidence for postlesion sprouting of this crossed perforant path (CPP), field potentials evoked by stimulation of the CPP increase some time after the lesion (Steward, Cotman & Lynch, 1974, Exp. Brain Res., 20, 45-66). Previously, all electrophysiological measurements of this pathway, in normal and lesioned animals, were obtained in acute experiments; until the present study, no electrophysiological measurements of CPP field potentials have been obtained in animals with chronic, indwelling electrodes. In the present experiment, the time course of lesion-induced increases in evoked responses of the CPP was studied over time in chronically implanted animals.

Twelve adult male Long-Evans rats (350 - 450 g) were implanted with bipolar, twisted-wire stimulating electrodes in the entorhinal area of the right hemisphere. A monopolar recording electrode was positioned in the hilus of the dentate gyrus in the left hemisphere. Averaged evoked potentials were collected daily, while animals were awake and unrestrained. After 7 to 14 days of baseline recording, the rats were given electrolytic lesions of the left entorhinal area. Daily electrophysiological measurements began again two days after the lesion, and were continued for each animal until postlesion changes in the evoked response reached stability. Significant increases in amplitude, of the monosynaptic CPP response, were found five days postlesion and in some animals as early as the fourth postoperative day. This is the earliest reported functional increase in this system, and indicates that chronic recording is probably more sensitive than acute techniques for detecting such changes. Response amplitudes continued to increase throughout the first postoperative week, and finally reached asymptote at around 8 - 12 days after the lesion. We interpret the response increase as the electrophysiological reflection of postlesion synaptogenesis that has been demonstrated anatomically (Steward et al., 1976).

Behavioral correlates of this time-dependent process are currently under study. Supported by a dissertation research award from Southern Illinois Univ. to T.M.R. and NSF grant BNS-8002251 to D.C.S.

288.3 QUANTITATIVE IMAGE ANALYSIS OF MOSSY FIBER SPROUTING IN THE SUPRAGRANULAR AND GRANULE CELL LAYERS OF THE DENTATE GYRUS FOLLOWING ENTORHINAL LESIONS IN ADULT RATS. J.R. West and S.L. Dewey, Dept. of Anatomy, University of Iowa, College of Medicine, Iowa City, IA, 52242.

Axonal sprouting is well documented in the mammalian brain. Typically, sprouting is a growth response by undamaged axons into a previously deafferented area. Mossy fibers, axons of dentate granule cells, project to the pyramidal cells in regio inferior of the hippocampus. A few collaterals are also known to project back to the molecular layer of the dentate gyrus. Following the removal of the entorhinal cortex (Laurberg and Zimmer, J.C.N., 200:433-459, 1981) or combined commissural and entorhinal lesions (Frotscher and Zimmer, J.C.N., 215: 299-311, 1983) mossy fibers sprout in the supragranular zone of the molecular layer and synapse on granule cell dendrites. To quantify the supragranular mossy fiber sprouting, we utilized an EyeCom II/PDP-11/34 image processing system. Coronal brain sections from six adult rats who received unilateral entorhinal lesions at least 30 days previously were photographed at 4X using Plus X Pan black and white film. The photographic negatives were backlighted and presented to the camera. The image was digitized and the contrast range enhanced. The range of dark brown to black mossy fiber staining in the hilus was used as a reference for determining the extrahilar mossy fiber staining in the dentate gyrus. The dentate gyrus was manually outlined and the areas of mossy fiber staining (both ipsi- and contralateral to the lesion) were quantified. There was a 160% increase in mossy fiber staining in the granule cell layer and supragranular portion of the molecular layer ipsilateral to the lesion compared to the nonlesion (contralateral) side. Based on the known termination of the perforant path projection, it appears that the removal of the entorhinal cortex results in mossy fiber sprouting into an area not deafferented by the lesion.

288.4 FAILURE OF BEHAVIORAL RECOVERY FOLLOWING UNILATERAL ENTORHINAL LESIONS. P.J. Best, V. Miller, J. Dudley, D. Wright & L. Leake. Dept. of Psychology, Univ. Virginia, Charlottesville, VA 22901.

Unilateral entorhinal lesions (UELs) result in deafferentation of the ipsilateral hippocampus, which is followed by sprouting of new afferents from the contralateral entorhinal cortex. Physiological evidence indicates that these new connections are functional. Also, behavioral recovery occurs over a time course that parallels the formation of new functional connections. Typically, immediately following UELs the behavior is indistinguishable from animals with bilateral entorhinal lesions (BELs), but in a few weeks it recovers to the behavior of intact animals, a most remarkable incidence of vicarious function.

The present study determines if such behavioral recovery occurs in a task more complex than those previously used. Rats were trained to forage for food in a 17-arm radial arm maze. One 45mg noyes pellet is placed at the end of each arm. An optimal foraging strategy is to visit all arms once and consume the food there without returning to any previously visited arms. Returning to a previously visited arm can be viewed as an error. Performance on this task was evaluated on the basis of the number of different arms visited in 17 traverses, and the number of different arms visited before the first error. Both measures were used in the present study.

Rats sustained either unilateral or bilateral electrolytic entorhinal lesions. The extent of the lesions was confirmed histologically. An unoperated control group and one UEL and BEL group were trained immediately following surgery. Other UEL and BEL groups were trained following a 27-day recovery period.

Neither UEL group showed significant recovery, nor did they learn the task to the level of the control rats. Further, the immediate UEL group did not differ from the delay UEL group at the beginning, middle, or end of training. However, both UEL groups were significantly superior in performance to both BEL groups at all stages of training.

In this task, unilateral entorhinal lesions result in a permanent debilitation of performance. Obviously then, the sprouting that occurs following a unilateral entorhinal lesion may be accompanied by a recovery on some behavioral tasks but recovery does not occur on other tasks which are apparently more complex or difficult.

288.5 SUBSTANTIA NIGRA LESIONS: PROJECTIONS AFTER CONTRALATERAL ELECTROLYTIC LESIONS STUDIED WITH ANTEROGRADE TRANSPORT. L.S. Jones and J.N. Davis. Neurology Research Laboratory, VA Medical Center, Durham, NC 27705 and Departments of Medicine (Neurology) and Pharmacology, Duke University, Durham, NC 27710.

Our laboratory has been studying central neuronal rearrangements that occur in response to injury. We are interested in the phenomenon of collateral sprouting: neurite outgrowth by intact neurons. We have investigated whether discrete, electrolytic lesions within one substantia nigra (SN) can elicit sprouting from the striatal-projecting neurons of the contralateral, intact SN.

We studied the anatomy of the SN projections in both lesioned and unlesioned rats with HRP-tagged wheat germ agglutinin (WGA) injected into the SN; we then looked for anterograde transport. The WGA-HRP was placed in the SN using a 1 ul Hamilton syringe in a dorsal approach; 80-100 nl was pressure injected over a period of 30-60 min., and the rat perfused 48 hours later. The brain was cut at 40 um and the HRP visualized using tetramethylbenzidine (TMB). We placed localized electrolytic lesions in the caudal SN of experimental animals by passing a 3 mA current for 10 sec. The lesions were confirmed histologically 7-11 days later when the rats were sacrificed. We injected the WGA-HRP into the SN contralateral to the lesion 48 hours prior to sacrifice.

The pattern of WGA-HRP transport in unlesioned rats largely confirmed known projections: ipsilateral labelling of striatum, globus pallidus, subthalamic nucleus, superior colliculus, midbrain reticular formation, peribrachial area, and ventromedial and lateral dorsal thalamus. Contralateral projections were seen only in the thalamus. The retrograde labelling confirmed known projections to SN. The lesions did not result in any change from the normal pattern of anterograde WGA transport. The technique's sensitivity to contralateral anterograde transport was confirmed by injection into entorhinal cortex that produced anterograde labelling of the contralateral hippocampus.

These data show that, using WGA-HRP with TMB, there is no change in the nigrostriatal projection within 7-11 days after an injury to the contralateral SN. Also, despite some electrophysiologic and biochemical evidence for a crossed nigrostriatal projection, our data confirm previous anatomical studies, which fail to demonstrate such a pathway. Furthermore, while there is work to suggest sprouting may occur along the crossed nigrothalamic pathway following 6-OHDA lesions, the present study using electrolytic lesions and anterograde transport does not provide evidence for sprouting in the nigrostriatal system.

Supported by NS-06233.

288.6 MEDIAL SEPTAL LESIONS DO NOT RESULT IN INCREASED VASCULARIZATION OF THE DENTATE GYRUS IN THE RAT. J. P. Chandler* and K. A. Crutcher (SPON: R. J. Mullen) Dept. of Anatomy, Univ. of Utah Sch. Med., Salt Lake City, UT 84132.

Lesions of the septohippocampal projection result in cholinergic denervation of the dentate gyrus (DG) and ingrowth of sympathetic axons from the superior cervical ganglion into the DG. We have shown that sympathetic axons do not normally occur over the rostral two thirds of the dorsal DG (Chandler and Crutcher, Anat. Rec. 205:33A, 1983). They must, therefore, grow a considerable distance to innervate the length of the dorsal dentate. These axons appear, by fluorescence microscopy, to accompany the penetrating vessels of the molecular layer. Whether they remain exclusively associated with blood vessels in the granule cell layer and in the hilus remains to be determined. The mechanism of ingrowth of these axons has not been elucidated. If they remain preferentially associated with blood vessels, then one possibility is that axons are passively drawn into the DG by neovascularization of the DG. In order to test this hypothesis, we counted the number of vascular profiles in the DG in normal animals and in animals that had received septal lesions.

Experimental animals were adult female Long-Evans rats that received lesions of the medial septum 4-12 months prior to sacrifice. Control animals were unoperated rats. Animals were perfused with aldehyde fixative and embedded in plastic. Sections were cut at two μm and mounted on glass slides. Using light microscopy, vascular profiles were counted in the molecular layer, granule cell layer, and hilus.

No statistical difference between numbers of blood vessels in control and experimental animals was found in any area. This suggests that there is no increase in vascularization of the DG following medial septal lesions in response to either the lesion or the ingrowth of closely associated sympathetic axons. Because neovascularization does not appear to occur, there must be another mechanism for ingrowth of the axons. We propose that sympathetic axons grow along existing blood vessels and enter the DG with penetrating vessels. This would require that axons grow some distance to reach the penetrating vessels that they accompany into the dentate parenchyma.

(Supported by PHS Grant NS 17131 and NRSA NS 07055.)

288.7 RECOVERY OF INTRASPINAL SUBSTANCE P AFTER SECTION OF THE CAT SCIATIC NERVE. A. Tessler, B. T. Himes, M. Murray and M. E. Goldberger. Depts. of Neurology and Anatomy, VA Medical Center and The Med. Coll. of Pennsylvania, Phila., PA 19129.

Sciatic nerve section depletes SP immunoreactivity (SPIR) in the cat dorsal horn and kills up to 19% of L7 dorsal root ganglion (DRG) cells (Tessler et al. '82). Transganglionic degeneration may therefore account in part for loss of intraspinal SPIR. Some of the intraspinal depletion may be due to decreased transport by axotomized but surviving DRG cells. Depletion by rhizotomy is followed by partial recovery, attributed to sprouting by SP-containing interneurons (Tessler et al. '81). SP loss by peripheral nerve section is long lasting (Jessell et al. '79). We used the PAP technique and RIA to determine whether intraspinal SPIR eventually recovers after peripheral lesion and thus is comparable to the recovery after rhizotomy. 60d after sciatic nerve section at midthigh, L7 DRG cell losses of 13 and 19% are seen, and intraspinal SP depletion is greatest. At 90-110d, 2 cats showed cell losses of 19%, but 5 cats have lost few (6-8%) or no cells. The cats with 19% cell loss contain only small amounts of finely staining SP reaction product in medial laminae I and II. Dorsal horns of cats which lose few or no L7 DRG cells contain more reaction product. The difference in L7 DRG cell loss may be a function of the numbers of DRG cells projecting in the sciatic nerve since different numbers of DRG cells are axotomized by a sciatic nerve lesion in different cats. Dorsal horn SP at 90-110d appears to be slightly greater than at 60d which is consistent with recovery but the anatomic variation in L7's contribution to the sciatic may indicate that the lesion produces intraspinal depletion only if it also produces DRG cell death. Thus the apparent recovery may only indicate cases in which there was little cell loss. If this were so, then SP levels in DRG and spinal cord should be parallel. We used RIA to measure SPIR in both the cord and L7 DRG of 2m and 6m survivors. SPIR in the dorsal quadrant is reduced by 30% at 2m but is normal by 6m, whereas SPIR in L7 DRG is reduced by 40% at 2m and at 6m. The persistent loss of SP in the ganglion indicates that these L7 ganglia are substantially axotomized by sciatic nerve section. The recovery in the L7 spinal segment (but not L7 DRG) at 6 months suggests that it depends on SP originating in the cord or other DRG's. Therefore, sectioning the peripheral processes produces effects resembling those following dorsal rhizotomy. Loss of DRG cells and reduced transport of SP by axotomized DRG cells may contribute to the depletion, and axonal sprouting of non-dorsal root, SP-containing cells may partly account for the recovery. Greater recovery after peripheral axotomy may be due to renewed transport of SP by DRG cells which are axotomized but survive.

Supported in part by the Medical Research Service of the Veterans Administration and NIH grant NS14477.

288.8 SUBSTANCE P IN THE INTERPEDUNCULAR NUCLEUS: 1. NORMAL DISTRIBUTION AND EFFECTS OF DEAFFERENTATION. R. Artymyshyn*, T. C. Eckenrode* and M. Murray. (SPON: K. F. Greif). Dept. Anatomy, The Med. Coll. of Pennsylvania, Phila., PA 19129.

The Interpeduncular Nucleus (IPN) is a midbrain structure that receives its major afferents from the medial habenulae (MHb) via the Fasciculus Retroflexus (FR). Among the habenular axons projecting to the IPN is a population of Substance P (SP) containing axons. The IPN has been subdivided into five subnuclei using cytoarchitectonic criteria (Ives, '71). The Pars Medialis (PM) forms the core of the IPN. The Pars Dorsalis Parvocellularis (PDP) forms a cap covering the dorsal aspect rostrally, beginning as a thin plate and thickening as it extends toward the caudal pole. The dorsal aspect of the caudal ¼ of the IPN is covered by the Pars Dorsalis Magnocellularis (PDM), an extension of the B8 serotonergic group of the Raphe. The Pars Lateralis (PL) forms a pair of lateral columns that are present throughout the IPN except at its rostral pole. These lateral columns extend from the base to about ½ the height of the nucleus. The distribution of SP in the IPN was compared in normal and FR lesioned Sprague Dawley rats. SP was visualized using the unlabeled antibody (PAP) technique. Lesions were placed unilaterally or bilaterally and animals survived 10 days to 3 months. In the normal IPN the PM contains sparse, punctate SP and scattered SP positive perikarya. The PDP and PDM contain a continuous band of punctate SP, moderate in density, that thickens as it extends caudally. The medial part of the PL contains very little SP; the lateral part contains very dense SP staining. After unilateral FR lesion, the SP in the rostral part of the PL is almost abolished, but caudally the decrease is confined to its lateral aspect. There is no visible decrease contralateral to the lesion in the PL. SP in the rostral part of the PDP decreases ipsilaterally. The caudal part of the PDP and PDM are not visibly affected. The PM does not show a decrease of SP but instead shows an increase in the amount of punctate staining and in the number of SP positive cell bodies. Bilaterally lesioned animals show a great decrease in staining in the PL and PDP, but no visible change in the PDM. There is again an increase in the amount of punctate staining and the number of SP positive cell bodies in the PM. These results confirm that most of the SP in the IPN is habenular in origin, but some of the SP is intrinsic. The habenular SP projection is primarily but not entirely ipsilateral. There is no visible replacement of SP to the PL after unilateral or bilateral lesions. The lesion induced increase in the amount of SP in the PM suggests compensatory sprouting. PM cells have been shown to project into the FR and may have been axotomized by the FR lesion, and the increase in staining may therefore represent a pruning phenomenon.

Supported by NIH grant NS16556.

288.9 SUBSTANCE P IN THE INTERPEDUNCULAR NUCLEUS: II. POSTNATAL DEVELOPMENT. T. C. Eckenrode*, W. B. Battisti*, R. Artymyshyn*, and M. Murray (SPON: R. P. Shank). Dept. Anatomy, The Med. Coll. of Pennsylvania, Phila., PA 19129.

There is evidence for both lesion induced sprouting and for failure of such sprouting in populations of axons in the Interpeduncular Nucleus (IPN). While some of these axon systems appear very early in development, others develop postnatally. In this study we describe the immunocytochemical localization and pattern of postnatal development of one of these systems, the Substance P (SP) system.

The IPN is a midline nuclear complex in which five subnuclei can be recognized (Ives, '71). Each of the subnuclei has a different distribution of SP. Lesion studies have shown that the SP arises from at least two sources. The primary source is the paired projection from the Medial Habenulae (MHb) via the Fasciculi Retroflexus (FR). Another likely source are the intrinsic SP neurons in the IPN. Sprague-Dawley rats were sacrificed at regular intervals from birth to 3 months. The distribution of SP in the IPN of these animals was examined using the unlabeled antibody (PAP) technique. Every third section was stained for Nissl substance. At one day postnatal the SP in the IPN is restricted to the Pars Lateralis (PL). The FR of these animals occasionally shows a moderate amount of SP staining. There is no SP staining in the Pars Dorsalis Parvocellularis (PDP), Pars Dorsalis Magnocellularis (PDM), or the Pars Medialis (PM) at this time and no visible SP containing cell bodies. At seven days postnatal, the SP in the PL appears denser than in the newborn. The PM and PDP still show no SP, but SP now appears in the PDM. The SP is sparse and diffuse, compared with the adult IPN. At 17 days postnatal the pattern of SP staining in the IPN becomes similar to that found in the adult. All of the subnuclei now contain SP and the regional distribution is the same as in the adult. At this time cell bodies can be visualized without colchicine. Considerably, more cell bodies can be visualized at this time than in the normal adult, a phenomenon which has been described by other authors (Hokfelt). We conclude that the habenular projection to the PL is present at birth and increases in amount postnatally. The habenular projection to the PDP appears later than the one to the PL. This suggests that the FR projection does not develop homogeneously. The intrinsic projection becomes apparent at 17 days postnatal and contributes to the SP in the PM. SP appears in the PDM by 10 days, and lesion studies have shown that this projection is not habenular in origin. This raises the possibility for a third source of SP in the IPN in addition to the habenular and intrinsic sources.

Supported by the Office of Mental Health of the Commonwealth of Pennsylvania and NIH grant NS16556.

288.10 SYNAPTIC REPLACEMENT IN DEAFFERENTED DORSAL HORN (LAMINA II) OF CAT. M. Murray, W. Battisti*, and M. E. Goldberger. Dept. of Anatomy, The Med. Coll. of Pennsylvania, Phila., PA 19129.

Light microscopic methods have provided evidence for collateral sprouting by intact pathways induced by dorsal rhizotomy. Degeneration methods have shown increased density of projection of descending pathways ipsilateral to the deafferentation (Goldberger and Murray, '74); immunocytochemistry and RIA of substance P (SP) have indicated sprouting or increased production of SP by spinal interneurons into laminae I and II depleted of SP by dorsal rhizotomy (Tessler et al. '82). These methods however do not provide direct evidence about either the time course of sprouting or the replacement of synaptic terminals. Therefore, we used quantitative EM methods to analyze the neuropil of lamina II in normal and deafferented cat. After unilateral, extradural lumbosacral dorsal rhizotomy (L_1-S_3), adult cats were allowed to survive from 2.5 to 6 days (4 cats) and 3-15 months (3 cats). Cross sections through the L_6 segment were prepared for EM. The unoperated side served as control. The cross sectional area of lamina II was measured planimetrically from projected drawings of 1μ sections stained with toluidine blue. There was no significant difference among control, acutely or chronically deafferented groups in the area of lamina II, indicating no net shrinkage. Stereological analysis was used to determine the percentage of the lamina occupied by axons, terminals, post-synaptic and non-neuronal structures. The area occupied by glia in deafferented animals is increased and this accounts for the maintenance of laminar area, compensating for the decrease in percent area occupied by myelinated axons (chronic) and axon terminals. Computer assisted morphometric analysis of synaptic terminal profiles permitted determination of mean area of profile, vesicle type and number of synaptic contacts per profile (multisynaptic index, MSI). In control lamina II, the majority of terminals are axo-dendritic and contain spherical vesicles; about 20% are large 'scalloped' terminals, making 2 or more synaptic contacts per profile (MSI > 1). This population represents nearly 40% of the total area occupied by terminals. After deafferentation, the terminals make axo-dendritic contacts and contain spherical vesicles but there are no 'scalloped' terminals, mean terminal area is less and the MSI is near 1. Estimates of number of terminals (area occupied by terminals/mean area per terminal) indicate no decrease in terminal number in either acute or chronic deafferented lamina II, despite the loss of the 'scalloped' terminals. The number of synaptic contacts is also preserved. We conclude that the synaptic terminals lost by deafferentation are replaced almost immediately by a population of small terminals with spherical vesicles.

Supported by NIH grants NS16556, NS16629 and NSF BNS 241775, and Office of Mental Health of the Commonwealth of Pennsylvania.

288.11 POSSIBLE LONG-DISTANCE SPROUTING IN THE CENTRAL NERVOUS SYSTEM OF THE GOLDFISH. R.L. Levine* (SPON: J. Marsden). Dept. Biology, McGill Univ., Montréal, Qué. H3A 1B1.

In a series of 10 goldfish (being used to examine a different problem) the right retina was removed. The animals were then divided into 3 groups. The first (N=3) had the right tectal lobe removed at the same time as the retinal removal was performed. The second group (N=4) underwent right tectal lobectomy 2 weeks after the retinal removal, while in the final group (N=3) the interval between the retinal removal and the lobectomy was 8 weeks. All animals received an intraocular injection (3 µL of 1% WGA-HRP) into the retinectomized eye at 4 weeks after tectal surgery. At this point the survival time after retinal removal for the various groups was: Group 1 - 4 weeks; Group 2 - 6 weeks; Group 3 - 12 weeks. Animals survived for 24 hours and were then sacrificed by perfusion and their brains were processed for HRP histochemistry. In unoperated, normal animals which had received intraocular WGA-HRP injections, no labeled cells were detected in the brain. By contrast, in the experimental animals, numerous neurons which had been axotomized by the tectal lobectomy were labeled by this procedure (thus indicating that they had grown into the retinectomized eye - see Levine, ARVO 1983). However, the observation of interest here is that many cells on the unoperated side of the brain were also labeled. These cells were nearly all located in one of three areas, the region immediately ventral to the nucleus rotundus, the dorsal pretectal region and the nucleus of the rostral mesencephalic tegmentum (NRMT - also called n.ruber by some workers). The mean, corrected (Abercrombie, 1946) number of cells labeled in the NRMT of each of the three groups was: Group 1 - 1.5; Group 2 - 11.0; Group 3 - 34.5. Since the cells of this nucleus are known to project only to the ipsilateral tectum, they were presumably not axotomized by the surgery and yet their labeling in this paradigm indicates that they too have extended axons into the retinectomized eye.

These data suggest that, in response to the tectal deafferentation afforded by retinal removal, cells of the NRMT (which normally have terminals in the tectum) sprout neurites which grow into the eye from which the retina was removed. This possibility is supported by preliminary observations in two animals in which the right retina was removed and no further surgery was performed. After 8 weeks the animals received intraocular WGA-HRP in the operated eye. In both animals cells were labeled in the NRMT corresponding to the deafferented tectal lobe (mean, corrected number of cells - 11.2).

288.12 SYNAPTIC REORGANIZATION IN RAT MOTOR TRIGEMINAL NUCLEUS FOLLOWING NEONATAL 6-HYDROXYDOPAMINE TREATMENT. L.M. Hemmendinger and R.Y. Moore. Dept. Neurology, SUNY-Stony Brook, Stony Brook, N.Y. 11794.

Neonatal 6-hydroxydopamine (6-HDA) treatment results in a marked loss of norepinephrine (NE) innervation to the telencephalon with hyperinnervation of the brainstem (Sachs and Jonsson, 1975). The increase in brainstem NE innervation is restricted to areas normally innervated by lateral tegmental and locus coeruleus NE neurons (Levitt and Moore, 1980), but it has been unclear whether the increased innervation observed following neonatal 6-HDA treatment represents an occupation of vacant synaptic space or a reorganization of synaptic architecture. The purpose of the present study was to investigate this problem by a quantitative ultrastructural analysis of the motor trigeminal nucleus (MTN). The MTN contains large motoneurons which receive a dense perisomatic innervation from lateral tegmental NE neurons. Four types of synaptic boutons have been described in the normal MTN by Card and Moore (1979). Boutons were characterized as having lucent (L) or dense (D) axoplasm with either spherical (S) or pleomorphic (P) vesicles. NE neuron terminals constitute an LS bouton population in the normal adult rat MTN.

Male pups received subcutaneous injections of 6-HDA on postnatal days 1 and 2. Littermate controls received vehicle injections, and animals were sacrificed at about 60 days of age. Brains were perfused by standard methods and ultrathin sections were photographed using a JEOL 100CX electron microscope. The number and distribution of each of the 4 bouton types forming axosomatic contacts were determined in sections through 5 neuronal perikarya from each of 4 control and 4 experimental animals.

The total number of boutons per 10 µm cell surface, or the percent of cell surface receiving axosomatic contacts (approximately 70%), was identical in each group. However, the number of LS and LP boutons per 10 µm cell surface increased 24% and 86%, respectively, over control levels. The number of DS and DP boutons decreased by 71% and 60%. These observations indicate that the increase in NE innervation of the MTN observed in fluorescence histochemical studies after neonatal 6-HDA treatment represents an increase in absolute number of NE boutons and suggests that this occurs because of a reorganization of synaptic terminals innervating the neuronal perikarya. It has not been determined whether LS boutons are the only NE terminals in the treated brains. If this is the case, these results indicate that the synaptic reorganization is more complex than a single increase in NE neuron terminals. The results of the study are considered with the view that the motoneuron cell surface may regulate the total number of axosomatic contacts on it but that the number of contacts formed by each specific afferent is not strictly regulated by the target cell. Supported by NS-17600.

288.13 PLASTICITY IN THE CAT TRIGEMINAL SYSTEM. L.E. Westrum and R.C. Dunn, Jr. (Spon: J.S. Lockard). Depts. of Neurological Surgery and Biological Structure, Univ. of Washington, Seattle, WA 98195, and Depts. of Anatomy and Neurosurgery, St. Louis Univ., St. Louis, MO 63104.

Plasticity as a result of deafferenting lesions is intimately dependent upon age and system. We have shown previously that one of the "critical periods" in synaptogenesis of the cat spinal trigeminal nucleus is about 3 days of age. We are testing the potential for reorganization of the cortico-trigeminal pathway following primary deafferentation by retrogasserian rhizotomy in 3 day old kittens (n=4) and in adults (n=3). Previously unoperated adults (n=4) with ablation of the sensorimotor cortex (including most of the coronal gyrus) show a somewhat variable degeneration pattern (Fink-Heimer and Nauta methods) but primarily to the contralateral subnuclei of the spinal complex. Degeneration ipsilateral to the cortical lesion is sparse to rare and this usually very medial in location. Similar cortical ablations in adults that had ipsilateral rhizotomies at 3 days of age show a moderate to heavy degeneration pattern that is widely distributed in the deafferented nucleus, especially in the periobex region; subnuclei caudalis, interpolaris and caudal oralis. Cortical ablation in adults that had adult rhizotomies (1 or more years earlier for removal of original degeneration) show a much reduced but somewhat variable pattern of ipsilateral degeneration. The results provide support for age-related plasticity in this system - either sprouting into the neonatally deafferented region or persistence of a neonatal pathway which may have otherwise been competitively eliminated with development of the primary afferents.

(Supported by NIH Grants NS09678, NS17111, NS15622 and DE04942. LEW is an affiliate of the CDMRC).

288.14 PARABIGEMINAL NUCLEAR PROJECTIONS IN HAMSTERS ARE ALTERED BY NEONATAL EYE REMOVAL. J.A. Stevenson. Department of Anatomy, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298

Previous studies have shown that contralaterally projecting axons of the rat parabigeminal nucleus (PBN) terminate in a restricted region of the superior colliculus (SC) but appear to be excluded from the dorsal lateral geniculate nucleus (dLGN). Removal of one eye at birth, a procedure known to produce enlarged ipsilateral projections from the remaining eye to the SC and dLGN, results in an enlargement of the crossed PBN projection to SC as well as the formation of a projection to the dLGN (Stevenson and Lund, J. Comp. Neurol., 207; 1982). This crossed PBN projection to the dLGN is likely to be the source of a population of aberrant axon terminals similar to those found in congenitally anophthalmic or neonatally enucleated mice (Cullen and Kaiserman-Abramof , J. Neurocytol, 5; 1976).

Recently, we have examined crossed projections of the PBN in hamsters using wheat germ agglutinin labeled with horseradish peroxidase as an anterograde tracer for both light and electron microscopic studies. The general form of the crossed PBN-SC projections in hamsters is similar to that of rats. The projection distributes within superficial tectal layers, is heaviest anteriorly and is absent from the posterolateral quadrant of SC. In apparent contrast to rats, the hamster does possess a crossed PBN-dLGN projection which terminates along the dorsolateral edge of the nucleus, and does not overlap the normal projection of the ipsilateral retina.

In animals which had left eyes removed at birth, the projections from left PBN to right SC were increased, as were the projections to the right dLGN. The geniculate projections in these animals extended throughout the nucleus but remained heaviest dorsally.

The detection of a crossed PBN-dLGN projection in normal hamsters may be due to the improved sensitivity of the tracing methods currently employed rather than a true species difference between rats and hamsters. Its presence suggests that previous descriptions of novel termination in dLGN of rat may more appropriately be regarded as examples of terminal sprouting by a preexisting PBN-dLGN projection in response to removal of retinal afferents.

288.15 SELECTIVE PLASTICITY IN DEVELOPING SUPRACHIASMATIC NUCLEUS INNERVATION FOLLOWING RETINAL DEAFFERENTATION IN THE RAT. M.F.Bernstein and R.Y. Moore. Department of Neurology, SUNY, Stony Brook, NY 11794.

Retinal input to the suprachiasmatic hypothalamic nucleus (SCN) is distributed to the ventral and lateral components of the caudal three-fourths of the nucleus. In the rat, the retinohypothalamic projection does not innervate the SCN until postnatal day 4 (P4) and ablation of input from one eye at P1 (Stanfield and Cowan, 1976) results in an expansion of innervation from the intact retina into the terminal field normally occupied by axons from the ablated retina. Partial destruction of the SCN results in a redistribution of retinal afferents into the caudal three-fourths of the remaining SCN, sparing the rostral pole (Mosko and Moore,1979). A secondary visual projection to the SCN arises from avian pancreatic polypeptide-like immunoreactive (APPLI) neurons in the lateral geniculate nucleus (Card and Moore, 1983). APPLI overlaps the distribution of retinal afferents in the SCN. This is distinct from Vasopressin-like immunoreactivity (VPLI) which is present in neurons and terminals located predominantly in the dorsomedial SCN and develops between P2 and P14 (DeVries et al., 1981). In the present study, the effect of eye enucleation at P2 on the development of APPLI and VPLI was examined. Bilateral orbital enucleations were performed at P2 and these animals and litter mate controls were sacrificed at P30-P40. Coronal sections through the SCN were prepared using the peroxidase-antiperoxidase indirect antibody method of Sternberger (1979).

In controls the rostral SCN contains APPLI in a dense plexus along the SCN-optic chiasm (OC) interface. At intermediate levels this plexus expands dorsolaterally creating a crescent-shaped plexus of reactive fibers more dorsally. In the caudal SCN APPLI is scattered laterally. Throughout the nucleus the dorsomedial SCN remains free of APPLI axons. There is no evidence for an extension beyond the lateral limits of the nucleus.

Following neonatal eye enucleation there is no change in the distribution of APPLI in the rostral SCN. At intermediate levels, the distribution of immunoreactive fibers extends dorsally to form a dense plexus in the dorsolateral quadrant of the nucleus. There is also a lateral extension beyond the nucleus to fill a wedge of tissue interposed between the SCN and the OC. In the caudal SCN there is no change from normal within the nucleus, but a few reactive fibers persist lateral to the nucleus. VPLI is not affected by the retinal deafferentation.

These data suggest that elimination of the retinohypothalamic projection to the SCN results in a selective increase and extension of a secondary visual projection of APPLI axons arising in the lateral geniculate nucleus.

Supported by NIH Grant NS 17600.

CATECHOLAMINES: BIOCHEMICAL CHARACTERIZATION I

289.1 BRAIN EPINEPHRINE-CONTAINING NEURONS: FUNCTIONAL ROLE IN REGULATINGMEDULLA-PONS NORADRENERGIC NEURONS. K. Braas, G. Vantini*, B.D. Perry, R. Gucchait, E. French, D.C. U'Prichard and J.M. Stolk* Maryland Psychiatric Res. Cntr., Univ. MD Sch. Med., Baltimore, MD 21228 and Dept. Pharmacol., Northwestern Med. Sch. Chicago, IL 60611

A primary distributional field of adrenergic neurons arising in C_1 and C_2 regions of the medulla is the nucleus locus ceruleus (Hokfelt et al, 1974); α_2-adrenergic binding sites are clustered in the same brain region (Young Kuhar, 1980). We previously observed that PNMT activity and epinephrine levels in medulla-pons of two inbred rats strains is correlated inversely with α-adrenergic receptor density (Perry et al, 1983), leading us to evaluate whether the relationship between PNMT and α-receptor binding sites has functional implications for regulation of noradrenergic neurons arising in the locus ceruleus. We assessed this potential relationship by treating F344 rats chronically with PNMT inhibitors (SKF 64139, LY 134046) or with α-receptor antagonists (prazosin, yohimbine); study measures were regional tyrosine hydroxylase activity (TH), norepinephrine (NE) levels and turnover, and radioligand binding. Chronic (6 d.) treatment with either PNMT inhibitor produced: (a) elevation of TH (b) elevation of α_2-receptor antagonist (^{3}H-rauwolscine) binding (c) modest increases in NE utilization in medulla-pons. Chronic yohimbine treatment caused similar changes in NE metabolism but prazosin treatment was without significant effect. Since these changes are seen in a brain area containing endogenous epinephrine they suggest that adrenergic neurons interact with medullary NE neurons through α_2-receptor mediated mechanisms. In brain areas devoid of endogenous epinephrine and PNMT (cerebellum, cortex, hippocampus), chronic PNMT inhibitor treatment had no effect on α-receptor radioligand binding or TH, but resulted in pronounced reductions of NE turnover and utilization in all regions tested. Prazosin was without effect, but yohimbine caused changes in TH and NE content different from those observed after PNMT inhibitor treatment. Thus, the effects of the PNMT inhibitors on NE turnover and utilization in distal NE terminal fields do not appear to be related to direct antagonistic activity on α_2-receptors. Tonic reductions in endogenous epinephrine "release" NE nerve cell bodies in medulla from α_2-receptor mediated suppression; the resultant local increase in noradrenergic neuronal metabolism (increased firing rate) effects distal adaptive changes in NE metabolism in terminal distribution fields that are secondary to reduced α_2-receptor stimulation by epinephrine in the medulla. (Supported by MN 32842, NS 15595, RSDA MH 00018 to JMS and NRSA MH 08834 to BDP).

289.2 SPECIFIC UP-REGULATION OF MEDULLARY/PONTINE AND HYPOTHALAMIC α-ADRENERGIC RECEPTORS AFTER PNMT INHIBITION. B.D. Perry, G. Vantini*, J.M. Stolk*, and D.C. U'Prichard. Dept. Pharmacology, Northwestern University Medical School, Chicago, IL 60611 and Maryland Psychiatric Research Center, Baltimore, MD 21228

Phenylethanolamine N-methyltransferase (PNMT) is the enzyme that converts norepinephrine (NE) to epinephrine (EPI). PNMT-positive/EPI-containing neurons originate in the C_1 and C_2 nuclei of the medulla and innervate spinal cord, medulla/pons (M/P) and hypothalamus (HYPO), playing key roles in many physiological processes including the regulation of blood pressure (Fuller, Ann.Rev.Pharmacol. Toxicol., 22: 31, 1982). Recently, we have reported intimate association of brain adrenergic neurons and α-, but not β-, adrenergic receptors (R) and an apparent "down-regulation" of M/P and HYPO α_1- and α_2-adrenergic Rs in Fisher 344 (F344) rats (high brain PNMT activity and EPI levels) relative to corresponding Rs in Buffalo (Buf) rats (5-8 fold lower brain PNMT activity and EPI levels than F344, Perry et al., Science, 1983) which was attributed to genetically-determined strain differences in PNMT activity and the resulting EPI levels (see Vantini et al., Soc. Neurosci., vol. 9, 1983). In order to confirm and extend our previous findings, we attempted to "up-regulate" M/P and HYPO α-Rs in F344 rats by "releasing" the α-Rs from tonically high levels of EPI by chronic treatment (6 days) with PNMT inhibitors (SKF 64139, LY 134046, DCMB) or α-antagonists (yohimbine, prazosin). Standard radioligand binding methods were employed, briefly (receptor: radioligand: blank, incubation time and temp). α_1: ^{3}H-prazosin (PRAZ): 100 µM (-)-NE, 40', 25°C or ^{125}I-HEAT (HEAT): 10 µM phentolamine, 60', 37°C. α_2(H): ^{3}H-p-aminoclonidine (PAC): 10 µM (-)-NE, 30', 25°C. α_2(L): ^{3}H-rauwolscine (RAUW): 100 µM (-)-NE, 120', 4°C. β: ^{125}I-iodocyanopindolol: 1.0 µM (-)-propranolol, 60', 37°C. Using single concentration assays, PAC, RAUW, and HEAT binding in F344 M/P and HYPO, but not cerebellum or cortex, was increased 30-100% (e.g., in M/P, PAC binding: control (C), 10.1; SKF, 31.3; LY, 15.1. RAUW binding: C, 6.3; SKF, 16.6; LY, 19.4. HEAT binding: C, 7.4; SKF, 12.3; LY, 10.4 and in HYPO, PAC binding: C, 21.5; SKF, 46.5; LY, 29.2. RAUW binding: C, 24.9; SKF, 51.8; LY, 39.7. HEAT binding: C, 27.3; SKF, 28.4; LY, 42.1. Values are means in fmol/mg prot, n=7-9 in M/P and 2-5 in HYPO). ICYP binding was not effected in M/P or HYPO by SKF or LY treatment and DCMB, which did not alter EPI levels under our conditions, did not alter binding. SKF treatment in Buf rats did not result in "up-regulation". α-Antagonist treatments did not effect binding in the same manner as PNMT inhibitor treatments. More detailed (saturation, nucleotide and ion effect) studies are in progress. The present results demonstrate specific regulation of M/P and HYPO α-Rs by EPI. This in vivo model will greatly facilitate study of brain adrenergic neurophysiology and the molecular pharmacology of α-R regulation. (MH 32842, NS 15595, RSDA MH 00018 and NRSA MH 08834)

289.3 LEVELS OF 3-METHOXY-4-HYDROXYPHENYLETHYLENEGLYCOL (MHPG) IN MOUSE BRAIN AS AN INDEX OF CENTRAL α_2-ADRENERGIC ACTIVITY. Wayne R. Cumiskey*, Richard A. Ferrari*, and Dean R. Haubrich. Dept. of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, New York 12144.

The α_2-adrenergic receptor antagonists yohimbine, mianserin, RX-781094 and RS-21361 were tested orally for their effects on MHPG levels in the brains of male Swiss-Webster mice. These receptor antagonists increase adrenergic neuronal firing by inhibiting the pre-synaptic α_2-receptors responsible for feedback inhibition of norepinephrine (NE) release at the synapse. The increased amount of NE released resulted in an increase in the brain level of the NE metabolite MHPG.

MHPG was determined by high performance liquid chromatography with electrochemical detection employing a C_{18} reverse phase column and a glassy carbon detector from Bioanalytical Systems and an automatic sample injector supplied by Waters Associates cooled in a Foster refrigerator. All of the MHPG in mouse brain was found to be in the unconjugated form. Recoveries in the 90% range were obtained in over 3000 samples analyzed.

The maximum increase in MHPG occurred 30 min after treatment with RS-21361 and 1 hr after treatment with the other three compounds. The potencies of each compound, determined from dose-response curves at the time of peak effect and expressed as the dose (mg/kg) necessary for doubling the brain MHPG concentration were: yohimbine (2.5) >mianserin (18) >RX-781094 (25) >RS-2136 (200). Prior subcutaneous injection of 0.3 mg/kg of the α_2-agonist clonidine prevented the increase in MHPG levels induced by all agents except RX-781094. The possibility that RX-781094 enhances NE release in brain by blockade of an adrenergic receptor that is not sensitive to clonidine is now being investigated.

These results demonstrate that compounds known to bind to α_2-receptors and to increase the rate of release of NE in vitro also stimulate the release of NE in vivo, as measured by the accumulation of MHPG within the brain.

289.4 A CONJUGATED FORM OF 3, 4 DIHYDROXYPHENYLGLYCOL IN POSTMORTEM HUMAN BRAIN. W. J. BURKE, G.M. BAUER* AND H. D. CHUNG*. ST. LOUIS VETERANS ADMINISTRATION MED. CENTER AND ST. LOUIS UNIV. MED. SCH., ST. LOUIS, MO. 63125.

We sought to determine whether a conjugated form of 3, 4 dihydroxyphenylglycol (DOPEG) was present in postmortem human brain. After hydrolysing the supernatant from a hippocampus homogenate in the presence of sulfatase and ascorbic acid, we injected a portion onto a high pressure liquid chromatography column with a dual electrochemical detector. A peak appeared with a retention time (r. t.) identical to that of DOPEG. However the peak height ratio (pk. rat.) measured with the detectors in parallel at 0.8 and 0.6 mv was 0.29 compared to 0.83 for DOPEG. After absorption and elution of the hydrolysed supernat from alumina a peak appeared which had the same r. t. as DOPEG and a pk. rat. of 0.86. To determine the source of the first peak, we subjected ascorbic acid without supernat to the same hydrolysis conditions. A peak appeared which had the r.t. of DOPEG but a pk. rat. of 0.29. This peak did not absorb to alumina. To determine a source for the DOPEG peak which appeared after hydrolysis, we injected untreated supernat onto the column. A peak appeared with r.t. 22 sec from DOPEG with a pk. rat. of 0.94. It did not correlate with any other known major metabolite of norepinephrine (NE). It was not absorbed to alumina, and could not be detected after hydrolysis. This peak thus fits criteria for a conjugated form of DOPEG. On the basis of recovery of DOPEG standard from alumina we calculated the concentration of conjugated DOPEG in tissue to be no less than 5.29 nmol/g of hippocampus. Thus it is a major metabolite of NE in postmortem human brain.

289.5 RESPONSIVENESS OF α_2-ADRENERGIC RECEPTORS IS DECREASED IN PLATELETS FROM DEPRESSED PATIENTS. J.C. Mitrius, M.Micunis, R.C. Arora, H.Y. Meltzer, and D.C. U'Prichard. Dept. of Pharmacology, Northwestern Univ.Sch. Med., Dept. Psychiatry, Univ. of Chicago, and Ill. State Psychiatric Inst., Chicago, Ill. 60611.

In order to gain an understanding of the biological basis of psychiatric diseases, we have been studying platelets as a readily accessable peripheral tissue from depressed patients and normal controls. Previously, we have reported that there was no difference in the affinity or number of α_2-adrenergic receptor binding sites in platelet membranes from non-medicated depressed patients using the selective antagonist [^{3}H]Yohimbine (YOH, Daiguji et. al. Life Sci., 1981, 29:2059). Other labs, however, have found in depressed patients an increased number of α_2-receptor sites using a radiolabeled agonist, [^{3}H]Clonidine (Garcia-Sevilla et. al.,Arch. Gen. Psychiatry, 1981, 38:1327). We decided to directly assess α_2-adrenergic receptor function in platelets from depressed patients and normal controls by examining a biochemical response to α_2-receptor activation, namely, (-)-epinephrine (EPI)inhibition of basal and PGE_1-stimulated adenylate cyclase activity. While there was no difference in basal, PGE_1 or NaF-stimulated activity, there was a selective attenuation of α_2-receptor mediated inhibition of basal and PGE_1-stimulated adenylate cyclase activity in platelets from depressed patients. The maximal extent of α_2-receptor mediated inhibition was not significantly different in the 2 groups, but IC_{50} values of EPI inhibition were right shifted several fold in platelets from depressed patients. The selective α_2-antagonists,YOH and rauwolscine, completely reversed EPI inhibition in both groups but appeared slightly more potent in antagonizing EPI inhibition in platelets from depressed patients. Since the full agonist EPI was less efficacious in adenylate cyclase assays, we decided to examine the nature of EPI competition of [^{3}H]YOH sites in platelet membranes from depressed patients and controls. Limited competition curves (5 points) of EPI against [^{3}H]YOH did not demonstrate any significant differences. However it is possible that the EPI competition curves did not detect the highest affinity state of the α_2-receptor. The high affinity state of the α_2-receptor is the state that is directly related to the coupling of the receptor to cellular functions. The observed functional subsensitivity appears to be contradictory to the increased[^{3}H]Agonist binding that occurs in depressed patients platelets. However, in other experiments with platelets and neuroblastoma cells, we have found evidence that suggests loss of coupling of the α_2-receptor is associated with an increase in the labeling of the highest affinity state. Abnormalities of platelet α_2- receptor binding sites may best be assessed by examining [^{3}H]EPI binding.

289.6 IN VITRO RELEASE OF ENDOGENOUS DOPAMINE: EFFECTS OF DEPOLARIZATION. Richard W. Keller, Jr., Karen L. Frayer*, Mary R. Petrus* and Michael J. Zigmond. Depts. of Biological Sciences and Pharmacology, University of Pittsburgh, Pittsburgh, PA.

We have studied the synthesis and release of endogenous dopamine (DA) from synaptosomes and slices prepared from rat striatum and incubated under basal or depolarizing conditions. Synaptosome-rich fractions were incubated at 37°C either in standard Kreb's phosphate buffer, pH 7.4, containing 125 mM NaCl, 1.25 mM $CaCl_2$, 1 mM $MgSO_4$, 5 mM KCl, 20 mM sodium phosphate buffer, 10 uM tyrosine, 10 mM glucose and 1 mM ascorbic acid, or in buffer modified to contain 55 mM KCl and 72 mM NaCl. In some cases ^{14}C-carboxyl labelled L-tyrosine was added and DA synthesis estimated from the evolution of $^{14}CO_2$. After 10 min the incubation was terminated by filtration (to measure DA release) or the addition of trichloroacetic acid (to measure DA synthesis). High KCl buffer resulted in a 4.6-fold increase in DA release. This rate greatly exceeded the capacity for DA synthesis. Consequently, while a significant increase in synthesis was observed (+23%), release was accompanied by an almost equivalent loss of DA from tissue.

In order to examine the pattern of KCl-induced DA release, striatal slices (350 u) were placed in a 250 ul chamber and perfused at 100 ul/min. In the presence of standard buffer, DA efflux was always highest in the first 3-min perfusate fraction and within 9 min had fallen to less than 1% of initial rate. This suggests that most of the spontaneous efflux observed in previous experiments without continuous perfusion had occurred early in the incubation, resulting in a large over-estimation of basal release rate. Consistent with this interpretation, when perfusion with high-KCl buffer was initiated, release increased 125-fold above the low, steady-state baseline. This rate immediately declined, however, reaching a level only 20 times baseline during 30 min of continual exposure to KCl. This decrease could not be explained by autoinhibition since it was not affected by haloperidol (100 nM). Moreover, a second exposure to high KCl buffer after 30 min in standard buffer resulted in only a small, transient increase in release. Thus, an exhaustion of releasable stores appeared to have occurred.

Collectively, these results suggest that depolarization with KCl is of limited usefulness in studies of DA release owing to the rapid, unphysiological rate of DA efflux and accompanying depletion of tissue stores which it elicits. In contrast, our present studies using electrical field stimulation to depolarize tissue slices suggest that in this way DA release can be elicited in a sustained manner without net loss of DA from tissue.

(Supported in part by a grant from the American Parkinson Disease Association and USPHS grants MH29670, NS16359 and MH30915.)

289.7 STIMULATION FREQUENCY DETERMINES PRECURSOR DEPENDENCE OF DOPAMINE SYNTHESIS AND RELEASE FROM STRIATAL NERVE TERMINALS. J.D. Milner and R.J. Wurtman. Lab. of Neuroendocrine Regulation, Department of Nutrition and Food Science, MIT, Cambridge, Mass. 02139.

The effect of tyrosine availability to dopamine neurons was studied using an in vitro preparation from rat striatum subjected to high and low frequency electrical field stimulation. Striatal slices (300 μm) were continuously superfused (0.5 ml/min) at 37°C with Krebs-bicarbonate buffer under constant 95%O_2/5%CO_2 (pH 7.4). Slices from the same animal were exposed to either tyrosine-free or tyrosine-enriched media (50 μM), and stimulated at 3 Hz or 20 Hz (60 mAmps, 2 msec, 1800 pulses) via Ag/$AgCl_2$ electrodes. The delivery of current was monitored continuously on an oscilloscope. Dopamine (DA) and its principal metabolite dihydroxyphenylacetic acid (DOPAC) were extracted from tissue and from effluent by alumina adsorption and quantitated by reverse-phase HPLC with electrochemical detection. Tyrosine levels were assayed by fluorimetry.

Dopamine release under the conditions described was frequency-dependent, calcium-dependent and blocked by tetrodotoxin (500 nM). When measured in the presence of an uptake blocker (nomifensine 10 μM) DA release was 1.0-2.0% of tissue content per stimulus period. The results described were obtained using drug-free media.

DA neurons stimulated at high frequency (20 Hz, 10/20 sec, 3 min) had up to 25% less DA and tyrosine ($p < 0.05$) when superfused with tyrosine-free media compared with DA content in tissue incubated with tyrosine, which did not differ significantly from control. There was no significant decline in tyrosine and DA levels in slices subjected to low frequency stimulation (3 Hz, 10 min). DA and DOPAC levels in the effluent were greater (2-4 fold) after stimulation at either frequency in the presence of tyrosine (50 μM) indicating the neuronal dependence on extracellular tyrosine for DA release. The frequency of stimulation did not affect total release of DA and efflux of DOPAC when summated over the stimulus period, indicating that DA release per pulse was probably constant.

The results suggest that extracellular tyrosine availability may be a critical factor in the acute regulation of dopamine synthesis and release from striatal nerve terminals during conditions of increased neuronal activity.

289.8 DYNAMICS OF DOPAMINE RELEASE IN THE CAUDATE NUCLEUS OF THE RAT INDUCED BY ELECTRICAL STIMULATION OF THE MEDIAL FOREBRAIN BUNDLE. Andrew G. Ewing*, Werner G. Kuhr*, W. Lowry Caudill*, James C. Bigelow* and R. Mark Wightman. Department of Chemistry, Indiana University, Bloomington, IN 47405.

Microvoltammetric electrodes in the caudate nucleus of the anesthetized rat can be used to monitor dopamine released after electrical stimulation of the medial forebrain bundle. The time resolution of the technique is sufficient to determine in vivo concentration changes on a time scale of seconds. Direct evidence for the identity of the substance released as dopamine was obtained both voltammetrically and pharmacologically. Administration of α-methyl-p-tyrosine abolishes the release of dopamine, although tissue stores of dopamine still exist, suggesting that there is a compartment for dopamine storage that is not available for immediate release. This compartment appears to be mobilized by amfonelic acid, since administration of this agent, after α-methyl-p-tyrosine, returns the concentration of dopamine released by electrical stimulation to 75% of the original amount.

The disappearance of dopamine from extracellular fluid following electrically stimulated release is very rapid and cannot be explained by dilution via diffusion processes. Postmortem analysis using liquid chromatography with electrochemical detection shows that dopamine released in this manner is metabolized to dihydroxyphenylacetic acid; however, neither substance is observed electrochemically in the extracellular fluid within seconds after the stimulation. In addition, inhibitors of neuronal uptake of dopamine, amphetamine (1.8 or 15 mg kg^{-1}) and benztropine (25 mg kg^{-1}), or dopamine metabolism, pargyline (150 mg kg^{-1}) or tropolone (100 mg kg^{-1}), do not significantly affect the rate at which dopamine disappears from extracellular fluid, although they can affect the amount released. These results suggest that dopamine cannot freely diffuse in the extracellular fluid because an extraneuronal uptake mechanism exists which leads to the metabolism of dopamine to dihydroxphenylacetic acid; however, dopamine can be observed during electrical stimulation of the ascending fibers because neuronal and extraneuronal uptake systems are unable to remove dopamine on these time scales.

289.9 PARTIAL LESIONS OF THE NIGROSTRIATAL PATHWAY: PHARMACOLOGICAL MANIPULATION OF TRANSMITTER SYNTHESIS AND RELEASE OF SURVIVING DOPAMINERGIC NEURONES. A. Enz*, F. Hefti and E. Melamed (SPON: J. Palacios). Sandoz Ltd., Preclinical Research, Basle, Switzerland, and Hadassah University Hospital, Jerusalem, Israel.

Injection of increasing quantities of 6-hydroxydopamine into the substantia nigra results in a dose-dependent reduction of the number of DA nigrostriatal neurons that survive the lesion. In animals with partial lesions, DA synthesis and release per surviving nigrostriatal neuron can be assessed by measuring the ratios of DOPA accumulation (after inhibition of AAAD)to DA and of HVA to DA. If the lesions reduce striatal DA levels to less than app. 40% of control values, these ratios are increased (Agid et al., Nature New Biol. 245, 150, 1973; Hefti et al., Brain Res. 195, 123, 1980). After mild partial lesions, a functional recovery occurs several weeks after the lesion (Dravid et al., in press). In the present study, we investigated whether the increased rates of synthesis and release after partial lesions represent the maximal rates or whether they are further enhanced by pharmacological manipulation.

Unilateral, partial nigrostriatal lesions were produced by injecting male rats with 6-10μg of 6-hydroxydopamine into the right anteromedial substantia nigra. These injections reduced DA levels in the ipsilateral striata to 2-20% of control values. On the lesioned sides, the striatal HVA/DA ratio was increased to 180% of the values measured on the contralateral side. Treating such animals with morphine (20mg/kg, 30min) further increased the HVA/DA ratio on the lesioned side to 350% of the ratio measured in unlesioned sides of saline-treated animals (=controls). On the unlesioned sides of morphine-treated animals, the HVA/DA ratio was increased to 140% of controls. Administration of haloperidol (2.5 mg/kg, 60min) increased the HVA/DA ratio in both lesioned and unlesioned striata to 550% of values measured in control striata.On the lesioned side of untreated animals, the ratio of DOPA accumulation to DA was increased to 400% of the values measured on the contralateral side. Administration of GBL (.75g/kg, 30min) further increased the DOPA/DA ration to 950% of control values. On the unlesioned sides, the ratio was increased to 280% of control values.

The results indicate that the enhanced rates of transmitter synthesis and release by DA neurons surviving partial nigrostriatal lesions do not represent maximal values but that they can be further enhanced by pharmacological manipulation. Extrapolating our results obtained on rat brains to the human brain, suggests that DA synthesis and release of the remaining population of DA neurones in parkinsonian brains can still be further accelerated. Drugs selectively enhancing DA synthesis and release without affecting postsynaptic DA receptors might therefore be therapeutically beneficial in the treatment of parkinsonism.

289.10 EFFECT OF ESTRADIOL AND PROLACTIN ON RAT DOPAMINE SYSTEMS: FURTHER CHARACTERIZATION. T. Di Paolo, M. Daigle* and N. Barden. Department of Molecular Endocrinology, Laval University Hospital Center, Québec G1V 4G2, Canada.

We have shown that chronic estradiol treatment increases the density of striatal dopamine receptors and decreases dopamine concentrations in ovariectomized as well as hypophysectomized rats. However, chronically high prolactin levels also elevate striatal dopamine receptor density. We have further investigated the effects of estradiol and prolactin on dopaminergic transmission with the following experiments. Ovariectomized female rats were treated with 17β-estradiol (.0002 to 100 μg b.i.d.) for 2 weeks. Doses of estradiol of 10 μg or higher significantly increased blood prolactin levels while striatal dopamine receptor levels, assayed by [^{3}H]spiperone binding, were elevated with estradiol treatments of .05 μg to 100 μg. [^{3}H]spiperone binding in the anterior pituitary expressed per mg of protein shows a biphasic dose-response to estradiol treatment, an increase being seen at low doses while a decrease is seen at doses 50-100 μg. These results indicate that the effect of estradiol may not be explained exclusively by increased prolactin levels since the effect of estradiol on dopamine receptors is seen at doses lower than those required to increase prolactin levels. The effect of chronic treatment with 17β-estradiol (.0002-10 μg) on dopamine, DOPAC and HVA concentrations was also studied in different brain nuclei and in the anterior pituitary. It is well known that dopamine inhibits the secretion of prolactin, and in accordance with the elevated levels of prolactin seen after 10 μg b.i.d. of estradiol or higher the anterior pituitary dopamine concentrations were decreased while the median eminence DOPAC and HVA concentrations were increased. In several brain nuclei of the mesolimbic and nigrostriatal dopaminergic systems a decrease in dopamine and its metabolites is seen after a treatment with 10 μg estradiol while lower steroid concentrations lead to non-significant changes or increases. Whilst we have previously demonstrated effects of constantly high prolactin levels on striatal dopamine receptors, in lactating rats very high prolactin levels are only attained periodically. In rats housed with their litter and lactating for 7-30 days, striatal [^{3}H]spiperone binding was significantly elevated compared to ovariectomized or intact female rats. Thus, repeated surges of prolactin as obtained in lactation can lead to increases in striatal dopamine receptor levels. In conclusion, estrogens can increase dopamine receptor levels at lower concentrations than those required to increase blood prolactin levels or decrease dopamine and its metabolites. Furthermore, high prolactin surges as obtained physiologically in lactating rats can also increase striatal dopamine receptors.

289.11 EFFECTS OF ESTRADIOL AND PROLACTIN ON INCERTOHYPOTHALAMIC DOPAMINERGIC NEURONAL ACTIVITY IN THE MALE RAT. K.J. Lookingland and K.E. Moore. Dept. of Pharm./Tox., Michigan State Univ., E. Lansing, MI 48824.

Incertohypothalamic dopaminergic (IHDA) neurons are located in the rostral periventricular and caudal dorsomedial regions of the hypothalamus. The activity of these DA neurons, like those in the major ascending nigrostriatal and mesolimbic systems, are regulated by DA receptor-mediated mechanisms (Lookingland and Moore, Fed. Proc. 42(4): 1155, 1983). In contrast, tuberoinfundibular dopaminergic (TIDA) neurons which are located in the mediobasal hypothalamus lack presynaptic autoregulatory DA receptors and are regulated by circulating concentrations of prolactin. The purpose of the present study was to determine if the activity of IHDA neurons, like that of TIDA neurons, is influenced by the administration of prolactin.

The activity of nigrostriatal, mesolimbic, TIDA and IHDA neurons was estimated by measuring the rate of turnover of DA (decline of DA after α-methyltyrosine, 250 mg/kg, i.p.) in brain regions containing the terminals of these neurons; i.e., in striatum, nucleus accumbens, median eminence and various hypothalamic nuclei, respectively. Estradiol benzoate (25 µg/kg/day x 3, s.c.), which markedly increased the concentration of prolactin in plasma, increased the rate of turnover of DA in median eminence, but not in striatum and nucleus accumbens. An intracerebroventricular injection of prolactin (10 µg/rat, 12 hr) mimicked the effect of estradiol on the turnover of DA in the same regions. Within the IHDA system, both estradiol and prolactin **increased** DA turnover in the dorsomedial nucleus, but surprisingly **decreased** DA turnover in the rostral regions of the IHDA system (i.e., periventricular, medial preoptic and preoptico-suprachiasmatic nuclei). Thus, on one hand IHDA neurons resemble the major ascending extrahypothalamic DA neurons in that they are directly responsive to DA agonists and antagonists, while on the other hand they differ from the latter neurons in that they are responsive to estrogen and prolactin. (Supported by NIH grant NS09174.)

289.12 THYROTROPIN RELEASING HORMONE AND ITS ANALOG MK-771 INCREASE THE EFFLUX OF DIHYDROXYPHENYLACETIC ACID BUT NOT 5-HYDROXYINDOLEACETIC ACID INTO CEREBROVENTRICULAR PERFUSATES OF UNANESTHETIZED RATS. J.A. Nielsen and K.E. Moore. Dept. of Pharmacol./Toxicol., Michigan State Univ., East Lansing, MI 48824.

Thyrotropin releasing hormone (TRH) is present in neurons throughout the brain where it may act as a neurotransmitter/neuromodulator. TRH and MK-771 (pyro-2-aminoadipyl-histidyl-thiazolidine-4-carboxamide) may influence the functioning of dopamine (DA) neurons. For example, TRH analogues increase the release of endogenous DA from rat brain slices (Sharp et al. J. Neurochem. 39: 1763, 1982). The present study evaluated the effects of TRH and MK-771 on the efflux of the DA metabolite, dihydroxyphenylacetic acid (DOPAC), and the 5-hyroxytryptamine (5HT) metabolite, 5-hydroxyindoleacetic acid (5HIAA), into lateral cerebroventricular perfusates of unanesthetized rats.

Male rats were implanted with permanent push-pull cannulas such that the tips were in the right lateral ventricles (Nielsen and Moore, Pharm. Biochem. Behav. 16: 131, 1982). After recovery from surgery the cerebroventricular system of each rat was perfused with artificial cerebrospinal fluid at a rate of 20 µl/min, and sequential 15 min perfusate samples were collected for 45 min before and 75 min after the i.p. or intracerebroventricular (i.c.v.) injection of TRH or MK-771. The concentrations of DOPAC and 5HIAA were analyzed by high performance liquid chromatography using a C_{18} µBondapak column coupled to an electrochemical detector set at +0.75 V.

TRH and MK-771 (20 mg/kg, i.p.) both increased the efflux of DOPAC into ventricular perfusate. Both drugs also produced a dose-dependent (2 and 20 µg/kg) increase in ventricular perfusate concentrations of DOPAC after i.c.v. administration; the effect of the higher dose was of greater magnitude and longer duration than the lower dose. Neither TRH nor MK-771 affected the perfusate content of 5HIAA. These results suggest that TRH and MK-771 affect the functioning of DA neurons without influencing the activity of 5HT neurons. (Supported by USPHS grant NS15911.)

289.13 OLFACTORY TUBERCLE AND HYPOTHALAMIC CATECHOLAMINE SYNTHESIS REGULATION: DIFFERENTIAL EFFECTS OF AMPHETAMINE AND CALCIUM CHELATION. Dean A. Haycock, Sharon E. Greenblatt*, Howard L. Askins* and Robert L. Patrick. Neuroscience Section, Division of Biology and Medicine, Brown University, Providence, RI 02912.

The present studies were carried out to test the hypothesis that the factors regulating tyrosine hydroxylase activity in the nerve terminal may vary according to brain region. Rat brain synaptosomal tyrosine hydroxylase activity was measured in the olfactory tubercle, an area in which dopamine is the predominant catecholamine, and in the hypothalamus, an area in which norepinephrine predominates.

The first set of experiments compared the effects of stimulant drug treatment on the two preparations. In the olfactory tubercle, assayed at a saturating tyrosine concentration, amphetamine produced a calcium-dependent stimulation of catecholamine formation. In the hypothalamus, however, amphetamine-induced synthesis stimulation was completely calcium-independent. Differences in amphetamine effects between these two brain areas were also observed when the tyrosine concentration in the incubation medium was varied. In the olfactory tubercle, amphetamine produced a biphasic effect--inhibition at low tyrosine concentrations and activation at saturating tyrosine concentrations. In contrast, hypothalamic catecholamine formation was stimulated by amphetamine at all tyrosine concentrations tested.

A second set of experiments compared the effects of the calcium chelator, ethylene glycol-bis(B-aminoethyl ether)N,N,N',N'-tetraacetic acid (EGTA), on catecholamine formation in olfactory tubercle and hypothalamic synaptosomes. Qualitative differences in responsiveness were observed; EGTA activated synthesis in the olfactory tubercle but had no effect in the hypothalamus. These data thus provide evidence for regional differences in synaptosomal catecholamine synthesis regulation and suggest that some differential drug effects may be related to differences in the effects of calcium on catecholamine formation. The results in the olfactory tubercle synaptosomes are similar to our previous observations in the predominantly dopaminergic striatal synaptosomal preparation, and are consistent with the concept that general differences in regulation may exist between dopaminergic and noradrenergic nerve terminals in the central nervous system.

(Supported by NIMH 31706, a Brown University BRSG grant from NIH and funding from the Grass Foundation).

289.14 ENDOGENOUS ASYMMETRIES AND CHANGES IN LEVELS OF DOPAMINE IN THE STRIATA OF SHAM-OPERATED AND MOTOR CORTEX INJURED RATS. Michael G. Boyeson, David A. Hovda, and Dennis M. Feeney, Departments of Psychology and Physiology, University of New Mexico, Albuquerque, NM (87131)

The present study measured levels of dopamine (DA) in the striata of sham-operated and right or left motor cortex lesioned rats. Analysis of DA levels by HPLC with electrochemical detection was performed on 36 animals at 48 h postcraniotomy or injury. Employing a t-test for correlated samples, a significantly greater amount of DA was found in the left striatum when compared to the striatum on the right in sham-operated controls. This result is consistent with recent findings of a greater number of DA receptors in the left striatum of normal rats (See Schneider et al., Neurosci Letters, 33, 281-284, 1982.), and may reflect a basic left-right asymmetry in the nigrostriatal pathway of the rat. Furthermore, the results suggest that if a naturally occurring lateralization exists in the striatum of the rat (or other areas), unilateral lesion studies using homologous contralateral structures as controls may be inappropriate. Additionally, in animals with either a unilateral right or left motor cortex suction ablation we found a significant bilateral drop in DA levels in both striata, indicating a widespread effect of unilateral lesions. An analysis of the effects of right vs left motor cortex injury on DA levels in the right or left striatum took into consideration the endogenous asymmetry in sham-operated animals. The analysis revealed that right motor cortex injuries significantly reduced DA levels in the right striatum compared to the left striatum. Our findings support and extend the hypothesis of Robinson (Science, 205, 707-710, 1979) damage to the right cerebral cortex in the rat produces an asymmetric change in catecholamine levels. Following left motor cortex injuries, no significant reduction in DA occurred in the left striatum compared to the right striatum that could not be explained by the basic asymmetry existing in sham-operated animals. Thus even though the left lesion reduced DA levels in the left striatum compared to the right striatum, the magnitude of the difference was no greater than would be expected of a population of rats with endogenous asymmetries. Supported by a grant from the Pennwalt Pharmaceutical Corporation.

289.15 COMPARATIVE EFFECTS OF REPEATED AND SUBACUTE ADMINISTRATION OF METHAMPHETAMINE ON METABOLISM OF BIOGENIC AMINES IN THE NEOSTRIATUM. T.M. Cook* and J.W. Gibb, Dept. Biochem. Pharmacol. & Toxicol., Univ. of Utah, Salt Lake City, UT. 84112.

Repeated toxic doses of methamphetamine (METH) produce a biphasic response on neostriatal tyrosine hydroxylase (TH) activity (Koda and Gibb, JPET, 105:42, 1973; Kogan et al., Eur. J. Pharm. 36:363, 1976). Neostriatal dopamine (DA) levels were significantly depressed at 36 hrs and remained depressed, although TH activity recovered by 72 hrs (Koda and Gibb, JPET, 185:42, 1973).

The present study examined the effects of repeated administration of METH (15 mg/kg, s.c.). The animals were dosed every 6 hrs and sacrificed at various time intervals after the initiation of treatment. TH activity was measured by the method of Nagatsu et al., (Anal. Biochem. 9:122, 1964). TPH activity was measured by a modified $^{14}CO_2$ -trapping method (Hotchkiss et al., Life Sci. 25:1373, 1979). Neurotransmitters and metabolites were quantitated using HPLC-EC (Nielsen and Moore, Pharmacol. Biochem. Behav. 16:131, 1982), measuring DA, DOPAC, HVA, 5HT and 5HIAA simultaneously. Repeated METH treatment caused a significant decrease in TH and TPH activity at 36 hrs (group A) and a progressive recovery to 80% of control by 72 hrs (group C). If administration of METH was discontinued after 5 doses, TH and TPH activities continued to decrease (group B). DA and serotonin levels were significantly decreased at 36 hrs and remained depressed in all groups.

	% Control		
	36 HRS	72 HRS	
	A	B	C
TPH	18±0.5	14±0.7	53±1
5HT	38±2	41±2	51±2
5HIAA	68±5	72±1	73±3
TH	46±1	30±2	80±1
DA	48±2	30±2	46±3
DOPAC	66±1	52±3	67±4
HVA	57±1	76±5	84±5

(Supported by USPHS Grant DA 00869)

289.16 TIME DEPENDENT REACTION OF RETINAL DOPAMINERGIC AMACRINE CELLS FOLLOWING 6-HYDROXYDOPAMINE TREATMENT. Gregory W. Maguire, Gail S. Tucker, D.I. Hamasaki* and Janie Rudolph*. Bascom Palmer Eye Institute, University of Miami Sch. of Med., Miami, FL 33101.

We have begun to follow the time course of retinal dopaminergic amacrine cell degeneration in adult cats and rabbits in response to the intravitreal injection of 50ul of 6-hydroxydopamine (6-OHDA; 1mg/ml) into one eye. The fellow eye received only the carrier solution (saline solution with ascorbate, 1mg/ml). Pargyline pretreatment preceded the first 6-OHDA treatment by 30 min. A second 6-OHDA injection followed two days later.

Four hours after the final 6-OHDA treatment, histofluorescence of endogenous dopamine was significantly reduced in processes within the inner plexiform layer (IPL), while the fluorescence in the somata was normal. These observations were consistent in both the cat and rabbit. At about 24 hours post-injection, the fluorescence of processes within the IPL was brighter than that of the controls. Beading of the processes was also apparent. About 48 hours post-injection the processes within the IPL were even more brightly fluorescent with very pronounced beading in both the cat and rabbit. We have previously reported a reduction in the high affinity uptake of tritiated dopamine 48 hours post-treatment in adult cats.

Electron microscope analyses are in progress to compare the ultrastructural effects with the biochemical effects. In one specimen 48 hours post-treatment the mid-peripheral retina was studied. Both plexiform layers appeared immature, and although the ribbon synapses looked adultlike, the conventional synapses were structurally immature.

Thus the retrograde reaction of retinal dopaminergic neurons following terminal lesions appears to have a time course similar to that seen in other central dopaminergic neurons.

289.17 PRETREATMENT WITH ORAL CDP-CHOLINE ENHANCES STRIATAL DOPAMINE (DA) RELEASE AND DIMINISHES STEREOTYPY IN RATS GIVEN APOMORPHINE PLUS HALOPERIDOL. J. Lopez G.-C.*, J. Agut*, P. Holbrook*, and R.J. Wurtman (SPON: C.E. Leprohon). Laboratory of Neuroendocrine Regulation, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.

Administration of choline chloride or of CDP-choline (Cytidyl Diphosphocholine) to adult male rats rapidly elevates striatal tyrosine hydroxylase activity, and could thus enhance striatal dopamine synthesis. We examined the effects of CDP-choline on striatal levels of the dopamine metabolites homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC), and on a behavior (apomorphine-induced stereotypy) known to be affected by dopamine release. Half of the animals received CDP-choline (100 mg/kg body weight) orally once a day; the others received the vehicle. On the fifth day of treatment, half of each group received haloperidol intraperitoneally; one hour later all animals received apomorphine (1 mg/kg, subcutaneously). Stereotyped behavior was assessed at 10, 20, 30, 40, 50 and 60 minutes after the administration of apomorphine. Behavior was rated by two independent blind observers, and numerical values were assigned to it according to the criteria of Montarano. After behavioral testing animals were sacrificed by decapitation, and the striata dissected and frozen in liquid nitrogen. HVA and DOPAC were analyzed by HPLC and electrochemical detection. The administration of haloperidol alone suppressed apomorphine-induced stereotyped behavior by 36% ($p<0.01$); in rats also pretreated with CDP-choline, the attenuation by haloperidol was increased, so that stereotypy was suppressed by 21% ($p<0.01$). Apomorphine alone decreased striatal HVA and DOPAC by 49% and 37% ; haloperidol administration reduced these decreases (to 164% and 62%, respectively), and CDP-choline pretreatment reduced them to a significantly ($p<0.01$) greater extent (to 223% and 100%). These data show that doses of CDP-choline known to elevate plasma and brain choline levels can enhance striatal DA release, and modify a DA-mediated behavior. Further studies will be needed to ascertain whether CDP-choline's neural effects involve more than its ability to serve as a choline source.

289.18 REGIONAL EFFECTS OF DSP-4 ON BRAIN NOREPINEPHRINE LEVELS AND TURNOVER. Mary Logue*, John H. Growdon* and Richard J. Wurtman. (SPON:P. Langlais). Laboratory of Neuroendocrine Regulation, M.I.T., Cambridge, MA 02139

DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine Hydrochloride)* is a systemically-active neurotoxin believed to damage central noradrenergic neurons. We examined the effects of its administration of norepinephrine(NE) levels and turnover in various brain regions. Groups of 5-6 adult male Sprague Dawley rats received the drug intraperitoneally in doses of 12.5, 25.0, or 50.0 mg/kg. Animals were killed 1 week later, and various brain regions were assayed by HPLC with electrochemical detection for NE and its metabolites 3-methoxy, 4-hydroxy phenylethyleneglycol sulfate ($MHPG-SO_4$), an index of NE turnover.

There were significant dose-related decreases in NE levels in the cerebral cortex, cerebellum, hippocampus, brain stem and hypothalamus after DSP-4 administration. There were similar significant decreases in $MHPG-SO_4$ levels in the cortex, cerebellum, hippocampus, and hypothalamus but not in the brainstem. NE turnover rates, estimated by calculating the tissue $MHPG-SO_4$/NE ratio, increased significantly in the cortex and in the hippocampus but not in other brain regions.

	HIPPOCAMPUS		
DSP-4 (mg/kg)	NE (ng/g tissue)	MHPG (ng/g tissue)	MHPG/NE Ratio
Control	308.7 ± 55	248.9 ± 42	0.83 ± .2
12.5	156.5 ± 21**	222.5 ± 18	1.52 ± .2
25.0	55.6 ± 12**	122.3 ± 26**	2.26 ± .2**
50.0	29.3 ± 3**	61.6 ± 13**	2.54 ± .5**

**P<0.01 (means ± S.E.M.)

These data indicate that DSP-4 is an effective noradrenergic neurotoxin damaging locus coeruleus neurons. Moreover they suggest that after its administration, the firing rates of some LC neurons, projecting to some brain regions, increase, thus increasing their turnover of NE.

*Kindly supplied to us by Dr. J. Scholthölt, Hoechst Laboratories, Frankfurt, West Germany.

289.19 **L-DOPA ACCUMULATION IS REDUCED IN STRIATUM AND RETINA OF THE DIABETIC RAT.** Madelyn H. Fernstrom, Patricia A. Grubb*, and John D. Fernstrom. Western Psychiatric Institute and Clinic, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

Among the metabolic disturbances occurring in diabetes is an elevation in the serum levels of leucine, isoleucine, and valine. These amino acids, plus tyrosine and tryptophan, enter the brain via competitive, carrier-mediated uptake. Brain concentrations of tyrosine and tryptophan are reduced in diabetic animals. The fall in brain tryptophan level is associated with a decrease in tryptophan hydroxylation rate. Because brain tyrosine levels are also reduced in diabetic rat brain, we examined wether this change might lower catecholamine synthesis and/or turnover. The retina was also studied, since dopamine may be a neurotransmitter in this tissue.

Male Sprague-Dawley rats (150-200 g) were made diabetic by a single, intracardiac injection of streptozotocin (65 mg/kg). Rats were used 2-3 weeks later; animals had free access to food and water and were maintained in light and temperature controlled quarters. For L-DOPA studies, rats were pretreated with NSD-1015 for 30 minutes. In other studies, untreated control and diabetic rats were killed, and striatal DOPAC and HVA levels determined. All measurements were made using HPLC coupled with electrochemical detection.

Variable	Control	Diabetes
Experiment 1		
Retinal Dopa	1.74 ± 0.11	1.07 ± 0.15*
Striatal Dopa	17.70 ± 2.00	12.00 ± 1.20*
Experiment 2		
Striatal DOPAC	7.70 ± 0.50	5.00 ± 0.20*
Striatal HVA	4.90 ± 0.30	3.40 ± 0.30*

[* P < 0.02 compared to control values. Data are means ± sem (n = 6), in ng/mg protein.]

As shown in the table, L-DOPA accumulation rate was reduced in the striata and retinas of diabetic rats. Striatal DOPAC and HVA levels were also decreased in diabetic animals, compared with normal rats. These data suggest that in vivo rates of tyrosine hydroxylase and dopamine synthesis (and/or release) are decreased in diabetic brain and retina. These changes may ultimately contribute to some of the nervous system abnormalities that develop in diabetes.

[Supported in part by a grant from the NIH (EY04980).]

289.20 THE PROPORTION OF D_2 DOPAMINE RECEPTORS IN HIGH AND LOW AFFINITY STATES DEPENDS ON THE AGONIST, CATIONS, GUANINE NUCLEOTIDES, AND TEMPERATURE. Masayuki Watanabe*, Susan R. George and Philip Seeman (Spon: P. Brawley). Dept. of Pharmacol., Univ. of Toronto, Canada.

D_2 dopamine receptors exist in two forms, with high (D_2^{High}) and low (D_2^{Low}) affinities for dopamine agonists. In the anterior pituitary it seems that D_2^{High} inhibits adenyl cyclase and prolactin secretion.

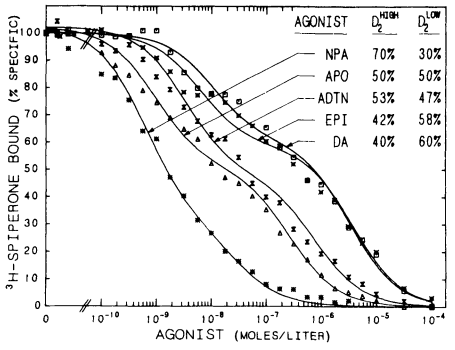
1) In keeping with reported low nanomolar dopamine concentrations in hypophyseal blood, we report, for the first time, K_D values for dopamine in anterior pituitary that are <10 nM.

2) The proportion of D_2^{High} and D_2^{Low} varied with the agonist present (Fig.): the % of receptors in the D_2^{High} state were highest for n-propylnorapomorphine (NPA) > dihydroxyaminotetralin (ADTN) = apomorphine (APO) > dopamine (DA) = epinine (EPI).

3) The presence of sodium ions (Na^+) increased the proportion of D_2^{Low} by ~50% while reducing the proportion of D_2^{High}. The effect of Na^+ was only apparent at temperatures of 23°C and 37°C and not seen at 4°C. The absolute K_D values for D_2^{High} and D_2^{Low} increased with increasing incubation temperature.

4) Guanine nucleotides alone also reduced the percentage of D_2^{High} and increased the K_D of D_2^{High}, but required the presence of Na^+ for complete conversion of all D_2 sites to D_2^{Low} at any temperature.

5) Longer incubations at the higher temperature also decreased the percentage of D_2^{High} without a loss in total receptor numbers. Thus, various factors modulate the proportion of D_2 receptors that are in the two affinity states and mediate interconversion between them.



CATECHOLAMINES: BIOCHEMICAL CHARACTERIZATION II

290.1 SUBCELLULAR SITE OF BIOSYNTHESIS OF THE CATECHOLAMINE-SYNTHESIZING ENZYMES. E.L. Sabban[1,2] and M. Goldstein[1], Depts. of Psychiatry[1] and Cell Biology[2], New York University Medical Center, New York, N.Y. 10016

The subcellular site of biosynthesis was examined for three of the catecholamine-synthesizing enzymes - tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT). TH and PNMT are predominantly soluble cytoplastic enzymes. While TH is unglycosylated, indirect evidence has been presented that PNMT, or a portion of it, may be glycosylated. DBH is a glycoprotein that is localized in chromaffin granules in the adrenal, and noradrenergic vesicles in neurons.

In order to examine the site of biosynthesis of these enzymes, free and membrane-bound polysomes were isolated from bovine adrenal medulla by an adaptation of the procedure of Ramsey and Steele (Anal. Biochem., 92:305-313, 1979) and used to prepare mRNA. The procedure yielded 2.6 times more mRNA in the free than membrane-bound fraction. Both fractions were active in directing protein synthesis in a wheat germ cell-free translation system with ^{35}S-methionine. Equal amounts of TCA-precipitable counts from translation of each mRNA were immunoprecipitated with antibodies to bovine DBH, or PNMT, or to rat TH. The specific immunoprecipitates were analyzed by SDS-gel electrophoresis and fluorography. Immunoprecipitation with antibodies to DBH, precipitated a translation product with apparent M_r=68,000 exclusively with membrane-bound mRNA. Newly synthesized PNMT (apparent M_r=32,000) and TH (apparent M_r=60,000) were predominantly (~80%) synthesized by free mRNA. These results indicate that similar to other secretory proteins, and to many membrane proteins, DBH is synthesized on membrane-bound polysomes. TH and PNMT are synthesized predominantly on free polysomes. The finding that PNMT is synthesized on free polysomes would indicate that PNMT is either unglycosylated, or glycosylated by a pathway other than the dolichol-pathway in the endoplasmic reticulum. Supported by Grants NINCDS 06801, NINCDS 18991, and NIMH-02717.

290.2 THE SYNTHESIS AND UPTAKE OF DOPAMINE AND SEROTONIN BY HUMAN Y79 RETINOBLASTOMA CELLS. M.A. Yorek* and A.A. Spector* (Spon: C.V. Gisolfi), Dept. of Biochemistry, Univ. of Iowa, Iowa City, IA 52242.

Y79 retinoblastoma is a human cell line derived from a tumor of the inner plexiform layers of the retina. The cultured cells have been previously shown to retain many neural characteristics, and we now find that these cells are capable of synthesizing the putative retinal neurotransmitters dopamine and serotonin. Tyrosine and tryptophan, the physiologic precursors of dopamine and serotonin, respectively, are taken up by both a high- and low-affinity transport system in Y79 cells. The K_m' and V_{max}' for the high-affinity uptake of tyrosine is 46.5 ± 8.4 μM and 3.38 ± 0.51 nmoles min^{-1} mg $protein^{-1}$, respectively. Phenylalanine is also taken up by a high-affinity system in Y79 cells. The kinetic parameters for phenylalanine uptake are very similar to those observed for tyrosine uptake, and it is likely that both of these amino acids are taken up by the same system. Separation of the catecholamines and indolamines by high performance liquid chromatography combined with electrical chemical detection showed that tyrosine added to the incubation medium is readily converted to 3,4-dihydroxyphenylalanine (DOPA) and, to a lesser extent, to dopamine. However, when DOPA is added as the precursor, a large quantity of dopamine is produced, as well as norepinephrine and 3,4-dihydroxyphenylacetic acid (DOPAC). Y79 retinoblastoma cells take up exogenous dopamine by both a high- and low-affinity system. The K_m' and V_{max}' for the high-affinity uptake system is 4.96 ± 1.05 μM and 9.35 ± 1.93 pmols min^{-1} mg $protein^{-1}$, respectively. The kinetic parameters for dopamine uptake by Y79 cells are similar to those reported for other retinal preparations where dopamine is suggested to function as a neurotransmitter. The K_m' and V_{max}' for the high-affinity uptake of tryptophan is 35.6 ± 4.7 μM and 5.15 ± 0.60 nmoles min^{-1} mg $protein^{-1}$, respectively. Tryptophan in Y79 cells is partially converted to 5-hydroxytryptophan and serotonin. When 5-hydroxytryptophan is added to Y79 cells, an equivalent amount of serotonin is produced. Serotonin is taken up by Y79 cells through only a single component, low-affinity system. The K_m' and V_{max}' for serotonin uptake are 3.41 ± 0.70 μM and 2.82 ± 0.64 nmols min^{-1} mg $protein^{-1}$, respectively. These studies show that a cell derived from the human retina synthesizes dopamine and serotonin from physiologic precursors. There is no high affinity uptake system for serotonin, however, in the Y79 cells. By contrast the kinetic analysis of dopamine uptake is consistent with its reported neurotransmitter characteristics in the retina. (This work was supported by NIH grants HL 14,230 and CA 09119).

290.3 CHARACTERISTICS OF ADRENERGIC RECEPTORS LINKED TO ADENYLATE CYCLASE OF CULTURED CEREBROVASCULAR SMOOTH MUSCLE. B. Wroblewska*, M. Spatz, N. Merkel* and J. Bembry*. Lab. of Neuropathology and Neuroanatomical Sciences, National Institutes of Health, Bethesda, Maryland 20205.

Adrenergic innervation of cerebral microvessels has been implicated in the regulation of cerebral blood flow (CBF) and permeability of blood brain barrier (BBB) . In support of this contention was the demonstration of catecholaminergic receptors linked to adenylate cyclase (AC) in the microvessels and in the cultured cerebrovascular endothelium. Since the control of CBF most likely is not only confined to the endothelium, we investigated the responsiveness of cerebrovascular smooth muscle cell AC to catecholamines.

In this report we will demonstrate that the cultured smooth muscle cells derived from dissociated cerebral microvessels contain β_2 and α_1 adrenergic receptors coupled to AC.

The pure smooth muscle cells used for these investigations consisted of 2nd-4th generation of cells cultured for 8-12 weeks. The preparation of cellular membranes and the incubation procedures for the measurement of AC activity in the presence and absence of the amines and their analogues were similar to those described for the cultured endothelium (Karnushina, Spatz and Bembry, Life Sci. 32: 1427, 1983).

The catecholamine analogues, zinterol and isoproterenol, were more effective in the stimulation of AC activity than epinephrine and norepinephrine. The EC_{50} (reflecting the relative ability of catecholamines to enhance cAMP formation) was 8.5×10^{-8}M for zinterol and isoproterenol, 7.5×10^{-7}M and 4×10^{-6}M for epinephrine and norepinephrine, respectively. When the selective antagonists for β_1 and β_2 receptors (β_1-type practolol and atenolol, β_1/β_2-type propandlol and β_2-type butoxamine) were tested against isoproterenol, epinephrine and norepinephrine stimulation of AC activity, the β_1 in contrast to β_2 antagonists were found ineffective. The α-blockers (phentolamine α_1/α_2-type antagonists) and yohimbine (α_2-type antagonist) alone or in the presence of propranolol had not significantly inhibited the catecholamine-induced enhancement of cAMP formation. On the other hand, prazosine (α_1-type antagonist) blocked the stimulatory effect of epinephrine and norepinephrine on AC system. Similarly, the α_2-agonist, clonidine, did not affect the catecholamines' stimulated AC activity while α_1 agonist, phenylephrine, induced a synergistic enhancement of norepinephrine production of cAMP.

The findings of β_2- and α_1-type adrenergic receptors in the cultured cerebrovascular smooth muscle provide additional support for the implicated involvement of adrenergic innervation in the regulation of CBF and BBB permeability.

290.4 EVIDENCE FOR RECYCLING OF ASCORBIC ACID DURING DOPAMINE-β-HYDROXYLATION IN CHROMAFFIN CELLS. F. S. Menniti and E. J. Diliberto, Jr.* Department of Medicinal Biochemistry, The Wellcome Research Laboratories, Research Triangle Park, NC 27709.

The final step in norepinephrine (NE) synthesis in neural tissue and adrenal medullary chromaffin cells is catalyzed by the enzyme dopamine-β-hydroxylase (DBH) which is localized entirely inside the catecholamine storage vesicle. The reaction requires $2e^-$ per molecule of NE formed which are supplied by ascorbic acid (AA). Previous work in our laboratory has shown that two AA molecules donate single electrons per β-hydroxylation cycle with the formation of two molecules of the free radical, semidehydroascorbic acid. Homogenates of chromaffin cells and brain tissue reduce semidehydroascorbic acid to AA via the mitochondrial outer membrane enzyme semidehydroascorbate reductase (SDR), providing a mechanism for reutilization of the cofactor.

In order to further study the relationship between AA and NE biosynthesis, we have measured the formation of NE from radiolabeled precursors at different intracellular AA concentrations in primary cultures of bovine adrenal medullary cells. AA levels were modified by using two properties of the cell cultures: 1) the decline of AA levels with time in culture via a first-order process with a $t_{\frac{1}{2}}$ of 31 hr (in contrast, enzyme activities, such as DBH, SDR, monoamine oxidase and glutamate dehydrogenase are stable); 2) the presence in chromaffin cells of an active transport system for AA capable of maintaining high intracellular levels of the cofactor when AA is added to the culture media. NE biosynthesis was studied by incubating the cell cultures for different periods of time with radiolabeled dopamine and/or tyrosine. Total catecholamines, tyrosine and AA levels were quantified by HPLC with electrochemical detection. HPLC fractions were also collected and radioactivity in each chemical species was determined by liquid scintillation spectrometry. The amount of NE synthesized per unit time was determined based on the measured specific activities of the precursors and the radioactive NE formed.

The results showed that the amount of NE synthesized did not diminish as AA levels declined with time in culture to as low as 0.1% of initial content. This corresponds to an intravesicular AA concentration of 0.1 K_m level of the cofactor for DBH. Furthermore, in depleted cells, the amount of NE formed over a 3-day period was as much as 287-fold higher than the total amount of AA available inside the cells. These results indicate that AA is efficiently recycled for NE biosynthesis, presumably by the mitochondrial SDR; however, we cannot at this time rule out the possibility of other electron donors for the DBH reaction in bovine adrenal medullary cells.

290.5 THE ROLE OF S-ADENSOYLMETHIONINE IN THE GLUCOCORTICOID REGULATION OF PHENYLETHANOLAMINE N-METHYLTRANSFERASE AND HYDROXYINDOLE O-METHYLTRANSFERASE. D. L. Wong, R. J. Hayashi* and R. D. Ciaranello. Dept. of Psychiatry, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Adrenal medullary phenylethanolamine N-methyltranferase (PNMT) and pineal hydroxyindoleamine O-methyltransferase (HIOMT) are protected against proteolytic degradation in vitro by their cofactor, S-adenosylmethionine (SAM). The in vivo degradation of these two biogenic amine enzymes is glucocorticoid regulated. This suggested that glucocorticoids might control in vivo degradation of the enzymes by modulating levels of SAM. Using a radioenzymatic assay developed in this laboratory, we have shown that hypophysectomy simultaneously decreases methyltransferase activity and SAM concentrations. Administration of dexamethasone, a synthetic glucocorticoid, reverses these effects. Moreover, SAM administration is just as effective as ACTH or dexamethasone in restoring PNMT and HIOMT activity.

We have also attempted to localize the specific loci of glucocorticoid action. Possible candidates are two enzymes responsible for SAM metabolism, methionine adenosyltransferase (MAT) AND S-adenosylhomocysteine hydrolase (SAHase). The former enzyme is responsible for SAM synthesis; the latter is responsible for the degradation of the metabolite [S-adenosylhomocysteine, (SAH)] generated during transmethylation. Hypophysectomy leads to concommitant decreases in PNMT, MAT, and SAHase activity in the adrenal gland. ACTH administration to hypophysectomized animals effectively restores the activity of all three enzymes in this tissue. In contrast, while hypophysectomy leads to a decrease in pineal HIOMT, MAT, and SAHase activity, neither dexamethasone or ACTH administration effectively elevates the activity of any of these enzymes.

These findings suggest that the glucocorticoid regulation of the in vivo degradation of PNMT and HIOMT mediated via the cofactor SAM may be controlled by separate mechanisms.

290.6 SPECIES DIFFERENCES IN PROPERTIES OF PHENYLETHANOLAMINE N-METHYLTRANSFERASE. D.H. Park and T.H. Joh, Lab of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Phenylethanolamine N-methyltransferase (PNMT) which catalyzes the reaction of norepinephrine to epinephrine in adrenergic neurons in brain and adrenal medulla, is known to exist in multiple forms in different tissues and species. We have recently found that inorganic phosphate activates the bovine adrenal form of PNMT, but does not activate rat adrenal enzyme, and that the differences are probably due to the presence of sialic acid moiety in bovine form of PNMT (Park et al., Neurosci. Abst. #252.11, 1982). In the present study, we sought to investigate other biochemical properties of PNMT in rat and bovine brain and adrenal medulla, including optimal pH for the enzyme activity, isoelectric point (pI) and the mechanisms for the enzyme activation by phosphate. PNMT was partially purified from brain and adrenal gland of rat and cow by passing the cytosolic proteins through a Sepharose 4B column. The optimal pHs for PNMT activity in phosphate buffer were: 8.2 for rat brain enzyme; 8.6 for rat adrenal enzyme; and 8.8 for enzyme from both tissues of cow. The pI of the enzyme from rat brain and adrenal, determined by chromatofocusing with the polybuffer exchanger, was 4.8. However, bovine brain and adrenal PNMT displayed several pIs ranging from 5.4 to 6.2. Although these differences exist, their primary structures seem to be similar. Antibodies raised against bovine adrenal PNMT cross-reacted with the enzyme from all sources, by Western blot and double immunodiffusion, and also inhibited activity of all enzymes. Thus differences in pIs and optimal pH of different forms are probably due to the differences in their tertiary structures which include the content of carbohydrate moieties. We next sought to investigate whether the brain forms of PNMT differ in the enzyme activation by phosphate from adrenal forms. Addition of 100mM phosphate in the enzyme assay mixture in Tris buffer increases PNMT activity in bovine brain by 2.4 fold, but decreases the activity in rat brain by only 25%. Bovine adrenal PNMT activity increases by 4.9 fold by phosphate, while rat adrenal PNMT increases only by 40%. Treatment of PNMT with neuraminidase has effect only on the bovine adrenal form of the enzyme by decreasing the activation from 4.9 to 2.5 fold. In contrast, bovine brain enzyme remains unchanged (2.4 fold) after neuraminidase treatment. The results indicate that multiple forms of PNMT exist in two different species. (Supported by Grants HL 18974 and MH 24285.)

290.7 SYNAPTOSOMAL TYROSINE HYDROXYLASE REGULATION BY DOPAMINE: LACK OF EVIDENCE FOR AN AUTORECEPTOR. David R. Compton* and Kenneth M. Johnson (SPON: P. Adams). Dept. of Pharmacology and Toxicology, Univ. of Texas Medical Branch, Galveston, TX 77550.

Reports in the literature have suggested the existence of an autoreceptor mechanism for regulating tyrosine hydroxylase (TH) activity in the dopamine (DA)-containing terminals of the striatum. In vitro experiments demonstrating this phenomenon are not always in agreement, even in identical paradigms. Studies demonstrating the existence of autoreceptor regulation of TH activity have utilized agonists other than DA. It is difficult to assess the role of autoreceptor regulation of TH activity by DA since feedback inhibition by DA occurs, subsequent to reuptake, via competition with the biopterin cofactor. By using pharmacological agents to minimize this mechanism, this study sought to determine the importance of receptor mediated control of TH activity by DA. The activity of TH in synaptosomes prepared from rat striatum was measured by a modification of the ^3H-H_2O release assay of Nagatsu (1964). TH activity was decreased by DA (IC_{50}=0.20μM). The effect of 1.0μM DA could be completely blocked by the addition of 3mM $DMPH_4$, an artificial cofactor, indicating that the primary mechanism of the DA effect is by classical feedback inhibition. DA-induced inhibition was not significantly altered by the presence of 1.0μM chlorpromazine (CPZ), sulpiride (0.1-10.0μM), or haloperidol (HAL)(0.01-1.0μM). These agents are representative of the three major classes of DA antagonists. Thus, there is no evidence of a receptor mediated event. Addition of 10.0μM nomifensine (NOM), a competitive uptake inhibitor, shifted the dose-response curve for DA inhibition to the right by approximately 10 fold. This inhibition was also unaffected by 1.0μM CPZ. This data suggests that DA inhibits TH activity directly, after reuptake, and not via a receptor, even at 10.0μM, a concentration 100 fold greater than that necessary to significantly inhibit TH activity.

DA (μM)	% Inhib ±S.D. (n)		DA (μM)	% Inhib ±S.D. (n)	
	cont	+CPZ		+NOM	NOM+CPZ
0.1	34±3(4)	42±10(3)	1.0	27±6(7)	24±5(3)
0.3	59±5(4)	69±10(3)	3.0	46±4(3)	52±7(3)
1.0	87±2(9)	88± 3(3)	10.0	78±7(4)	83±4(3)
IC_{50} (μM)	0.20	0.14		3.06	2.78

This report strongly suggests that autoreceptors are not involved in the physiological regulation of TH by DA in the striatal synaptosome. This work was partially supported by ADAMHA grant DA-02073.

290.8 THE RAPID ACTIVATION OF CYCLIC AMP-DEPENDENT PROTEIN KINASE TYPE I IN THE ADRENAL GLAND BY ELECTROCONVULSIVE SHOCK (ECS) AND ITS RELATIONSHIP TO TYROSINE HYDROXYLASE ACTIVITY. Joseph M. Masserano and Norman Weiner. Dept. Pharmacol., Univ. Colo. Med. Center, Denver, CO 80262.

We have shown previously that various stresses, including decapitation (Mol. Pharmacol. 16: 513, 1979) and electroconvulsive shock (ECS) (Science 214: 662, 1981), will rapidly activate tyrosine hydroxylase in the rat adrenal gland. In the present study we have examined the effects of ECS on the activation of cyclic AMP-dependent protein kinase (total, type I and type II) in the adrenal medulla. Rats were shocked with a 300 mA current applied transorbitally for 0.2 seconds. This produces a consistent tonic-clonic seizure lasting approximately 30 to 50 seconds. Following ECS, the animals were anesthetized with either pentobarbital or halothane at various time intervals and the adrenals were removed surgically under anesthesia. Adrenal medullae were dissected out, rapidly frozen, homogenized and centrifuged (5 min). Initially, the supernatant was assayed for tyrosine hydroxylase activity and cyclic AMP-dependent protein kinase activity. Approximately an 80% activation of tyrosine hydroxylase was obtained at 2 and 5 minutes following ECS. By one hour tyrosine hydroxylase activity had decreased to 20% above control values. Utilizing the method of Corbin et al (J. Biol. Chem. 248: 1813, 1973) for analyzing protein kinase activity, the ratio of protein kinase activity (-cAMP/+cAMP) in the adrenal medulla five minutes following ECS, increased from 0.33 to 0.51, and reached maximal levels (0.94) by 20 minutes.

Two minutes following ECS, adrenal medullae from rats were removed and examined for type I and type II protein kinase activities. The supernatant prepared from 16 adrenal medullae (control or ECS treated) were placed on DEAE-Sephacel columns (2.5 ml). Free catalytic subunit, protein kinase type I and type II were eluted using a 20 mM to 250 mM NaCl gradient. Free catalytic subunit eluted in the 20 mM NaCl wash buffer. Type I protein kinase eluted at 50 mM to 100 mM NaCl and type II protein kinase eluted at 110 mM to 160 mM NaCl. A decrease in protein kinase activity in either peak I or peak II with a corresponding increase in the activity in the free catalytic subunit peak reflects a dissociation, and consequently, an activation of the protein kinase holoenzyme. Two minutes following ECS, type I protein kinase was approximately 70% to 90% activated, whereas type II protein kinase was only slightly affected (15 % activated). In all assays, protein kinase activity was measured in the presence of 4 mM EGTA to inhibit calcium dependent kinase activities. It thus appears that the activation of type I protein kinase precedes the activation of type II protein kinase in the adrenal medulla following a single ECS. These results suggest a possible association of protein kinase type I activation with activation of tyrosine hydroxylase. This research was supported by USPHS grants NS 07927, NS 09199, and AA 03527.

290.9 TYROSINE HYDROXYLASE: STABILITY OF THE PURIFIED RAT PHEOCHROMOCYTOMA ENZYME. H. Wilgus*, K.E. Vrana* and R. Roskoski Jr. Department of Biochemistry, LSU Medical Center, New Orleans, LA 70119.

Tyrosine hydroxylase is the rate-limiting enzyme in the biosynthesis of the catecholamines. The activity of tyrosine hydroxylase is increased by cAMP-dependent protein phosphorylation. Tyrosine hydroxylase, purified from a transplantable rat pheochromocytoma, is phosphorylated by the purified catalytic subunit of the cAMP-dependent protein kinase. Although phosphorylation increases the activity, it decreases the stability of the enzyme. This destabilization is reflected as an increase in thermal lability. Moreover, the loss of enzyme activity is accelerated by the presence of a number of agents. Reduced pterin molecules (tetrahydropterins, tetrahydrofolate) will accelerate inactivation at nanomolar concentrations. Oxidized pterins, on the other hand, do not mediate inactivation. General reducing reagents (0.8 mM NADPH, 1 mM beta-mercaptoethanol, 1 mM dithiothreitol) do so at higher concentrations. *Accelerated inactivation is observed* when the reducing compounds are incubated with tyrosine hydroxylase (under phosphorylation conditions) for varying periods of time prior to enzyme activity determination. Inactivation is not associated with a change in the apparent molecular weight of the enzyme subunit - ruling unlikely a protease-mediated mechanism. Furthermore, there is no change in the apparent phosphorylation state of the protein. The latter finding suggests that hyper- or hypo-phosporylation of the enzyme in the presence of the inactivator does not occur. We hypothesize that phosphorylation of tyrosine hydroxylase reduces its inherent stability. The presence of reducing reagents (most notably the reduced pterin co-substrate) dramatically increases the loss of enzyme activity. This work was supported by USPHS Grant NS-15994 and Training Grant T32HL07098.

290.10 IDENTIFICATION OF THE PHOSPHOPROTEIN PHOSPHATASE ACTIVITY ATTENDING TYROSINE HYDROXYLASE IN ADRENAL CHROMAFFIN CELLS. R.J. George, J.W. Haycock and J.C. Waymire. Dept. Neurobiology & Anatomy, University of Texas Medical School, Houston, TX 77225.

Phosphorylation-dephosphorylation of tyrosine hydroxylase (TH), the initial and rate-limiting enzyme in the biosynthesis of catecholamines, provides a rapid and sensitive mechanism by which the enzyme's activity can be coupled to stimulus-dependent secretory processes. The phosphorylation-dependent activation of TH has been extensively studied in several systems. In bovine adrenal chromaffin cells, we have recently demonstrated that the stimulus-dependent phosphorylation of TH can be accounted for by a calcium-dependent protein kinase activity endogenous to the cells. Little, however, is known about the enzymes regulating the dephosphorylation of TH. Yamauchi and Fujisawa[1] have demonstrated a Mg^{2+} dependent loss of ^{32}P from TH in homogenates of bovine adrenal medulla phosphorylated in vitro by cAMP with a concurrent deactivation of the enzyme and Lazar et al.[2] have shown the dephosphorylation of bovine adrenal TH by *extracts of various rat tissues.*

The apparent heterogeneity of phosphoprotein phosphatases (PPO_4tase)--as judged by molecular weight, substrate specificity, and cation requirements--has made identification of specific PPO_4tase activity a complex task. Cohen and coworkers have proposed a general classification of serine and threonine PPO_4tases which divides the enzymes into two types (Types 1 and 2) based on their sensitivity to specific protein inhibitors and further subdivides the second type into Types 2a, 2b and 2c based on substrate specificity and cation requirements. Using this scheme of classification we have attempted to identify the PPO_4tase activity responsible for the dephosphorylation of TH phosphorylated by the calcium-dependent protein kinase activity endogenous to the chromaffin cells.

TH was phosphorylated in 100,000 x g supernatant fraction of the chromaffin cells by addition of calcium in the presence of Mg^{2+} and [gamma-^{32}P]ATP. The supernatant was gel filtered to terminate the phosphorylation reaction and to remove low molecular weight effectors of the endogenous PPO_4tases. At 37°C, about 80% of the ^{32}P associated with TH was lost within 15 min. The I_2 inhibitor protein inhibited this loss of ^{32}P from TH whereas Mn^{2+} and Mg^{2+} (but not Ca^{2+}) accelerated dephosphorylation. These characteristics are consistent with those of Type 1 PPO_4tase as described by Cohen and suggest that a Type 1 PPO_4tase could be involved in the regulation of TH in the chromaffin cells.

[(1) J. Biol. Chem. 254, 6408 (1979); (2) Neurochem. Int. 5, 107 (1983)]

290.11 EFFECTS OF INTRAVENTRICULAR XYLAMINE ON BRAIN MONOAMINES. M.A. Geyer, J. Gordon* and L.M. Adams*. Dept. of Psychiatry, Univ. of California, San Diego; La Jolla, CA 92093.

The neurotoxin 6-hydroxydopamine (6OHDA) has been useful in the evaluation of the functions of brain catecholamines (CAs). While selective depletions of dopamine (DA) can be produced with 6OHDA by protecting norepinephrine (NE) neurons with desipramine, selective NE depletions are more difficult to obtain since inhibitors of DA uptake have not been identified. Hence, the benzylamine neurotoxins such as xylamine (XYL), which deplete central NE without affecting DA, may enable assessments of the effects of central NE depletions without confounding of concomitant DA depletions. Given systemically, XYL produces a transient depletion of peripheral NE and a prolonged depletion of brain NE. While DA levels remain normal, brain serotonin (5HT) is reduced. For behavioral evaluations of the role of central NE neurons, the peripheral depletion produced by systemic XYL is a major complication. Therefore, we have examined the effects of intraventricular XYL in rats.

Male Sprague-Dawley rats (250-300 g) received bilateral stereotaxic injections of saline or XYL (10 ul/side) into the lateral ventricles. A dose of 50 ug XYL per rat was used, based on pilot studies with doses of 1-75 ug. After regional brain dissection, monoamines were assayed using standard methods for HPLC/EC. CAs and 5HT were assayed separately.

With ether anesthesia and 10 days post-surgery, 50 ug XYL produced a 65% decrease in hippocampal NE and only a 36% reduction in hypothalamic NE, confirming the regional differences reported for systemic XYL. However, hippocampal 5HT was also depleted by 73%. With nembutal anesthesia, hippocampal NE was depleted by only 43% and 5HT by 53% following 50 ug XYL. Due to the 5HT depletion, animals were pretreated with 10 mg/kg fluoxetine (FLU), a 5HT uptake inhibitor, in an attempt to protect 5HT neurons. With FLU, hippocampal NE was still depleted (47%), while the 5HT depletion was reduced to only 35%. Since smaller NE depletions were found with nembutal, subsequent studies used ether. Since 10 mg/kg FLU only partially protected 5HT neurons, another study used 20 mg/kg FLU and 8 post-operative days. With no FLU, 50 ug XYL reduced hippocampal NE by 60% and 5HT by 69%; with 20 mg/kg FLU, the NE depletion was 58% and 5HT was reduced only 5%. Caudate DA levels were not affected. With 15 mg/kg FLU, NE was reduced by 60% at 8 and 20 days, while 5HT was reduced by 14% at 8 days and 19% at 20 days.

These results indicate that intraventricular XYL does deplete both NE and 5HT, while having little effect on DA. FLU appears to protect brain 5HT neurons from XYL in a dose-dependent manner, with 20 mg/kg offering complete protection.

Supported by NIDA Grant DA02925. We thank Dr. Arthur Cho, UCLA for xylamine and Lilly for fluoxetine.

290.12 USE OF PRECOLUMNS TO INCREASE SELECTIVITY IN LIQUID CHROMATOGRAPHIC ANALYSIS OF BIOGENIC AMINES. E. F. O'Connor*, S. R. Winternitz and S. Oparil*. Cardiovascular Research and Training Center, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

Reverse phase ion pair liquid chromatography with electrochemical detection has become the method of choice in the analysis of biogenic amines and their metabolites in tissue. For most applications sample preparation is needed prior to analysis. These procedures are time consuming and may be a source of error.

Recently, Koch and Kissinger (1981) described a precolumn modification of an existing chromatograph which reduces sample handling while increasing analyte concentration and sample clean up. However, the hardware necessary to implement their precolumn system is expensive and not readily available to most laboratories.

Using the configuration they suggested, we have developed a similar method employing precolumns but without the additional hardware. In our system an Upchurch Scientific precolumn replaces the sample loop in a Rheodyne 7125 valve. Samples are loaded onto the precolumn with a gas tight Hamilton syringe. The valve may then be switched to inject and the sample is backflushed onto the analytical column using a suitable mobile phase. To make full use of the precolumn a 200 to 300µl volume of .001M pH 3.0 buffer is used to rinse the precolumn following the addition of the sample but before the valve is switched to inject. When a cation exchange resin is in the precolumn, acidic (DOPAC, homovanillic acid, 5-hydroxyindoleacetic acid) and neutral (DOPEG, MHPG) sample components are flushed out to waste, while the amines noradrenaline, dopamine and serotonin are retained. When the valve is switched to inject, these cations are eluted onto the analytical column. We have found that the use of cation exchange resin in a precolumn greatly reduces sample handling normally associated with biogenic amine analysis, while recovery of amines from standards or from brain tissue is approximately 96%. In addition, the elimination of interfering compounds allowed us to reduce chromatographic run time from 40 to 16 minutes without losing resolution. Finally, the sensitivity of the analysis is improved both from the increase in peak height and from the precolumn's ability to concentrate the analytes. The selectivity of the assay was modified in a predictable way when anion exchange or reverse phase resins were substituted.

The use of precolumns as described represents a simple modification of existing chromatographs. The implementation of this procedure is inexpensive and will eliminate the need for off-column sample extraction. Further, the technique increases separation efficiency while reducing errors from sample handling.

290.13 A SIMPLE TECHNIQUE FOR THE IN VITRO MEASUREMENT OF ENDOGENOUS DOPAMINE RELEASE. Eddie Castañeda*, Jill B. Becker, and Terry E. Robinson (SPON: Henry Buchtel). Dept. of Psychology, University of Michigan, Ann Arbor, MI 48109.

A sensitive yet simple technique to study endogenous dopamine (DA) release from striatal tissue fragments in vitro is described. Chambers to hold the tissue fragments are inexpensively constructed from the barrel of 1cc syringes with gaskets and rubber stoppers manufactured from the rubber tips of the plunger. 18 gauge stainless steel tubing is used for the inlet and outlet tubing. Experiments were conducted with these chambers to determine: (1) Optimal flow rate; (2) Dose-response for K+-stimulated DA release; and (3) Dose-response for amphetamine (AMPH)-stimulated DA release.

Following decapitation, rat striatal tissue was dissected and cut into 1 mm^2 fragments on ice. The striatal fragments were placed into chambers that were immersed in a 37° C water bath. Perifusion with a modified Krebs-Ringer phosphate buffer (pH 7.4) started immediately. Following a 1 hr equilibration period, effluent samples were collected on ice in 5-min intervals. Samples were assayed using HPLC with electrochemical determination. (1) Flowrate (50, 100 & 150 µl/min) had very little effect on spontaneous DA release from striatal fragments. Thus, for all subsequent experiments a flowrate of 100 µl/min was used. (2) A dose response relation for 60 mM K+-stimulated DA release was determined using 30 sec, 1, 2, 3, 4, and 5 min infusion times. A significant dose response relationship across pulse intervals was found ($F=2.9$; $p<0.04$). On the basis of this experiment, it was determined that a 2.5 min infusion of 60 mM K+ produced an easily detectable, reliable, yet not maximal amount of DA release. This dose of K+ was used in the subsequent experiment to examine the effects of 3 sequential exposures to K+. Although the amount of DA released in response to K+ declined significantly over the 3 peak responses ($F=14.6$; $p<0.001$) there was still a highly significant and reliable release of DA even following 125 min of perifusion and a total of 7.5 min exposure to 60 mM K+, indicating the tissue continued to be viable. (3) The third set of experiments was conducted to establish a dose response to AMPH exposure. AMPH was infused into the chamber at 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, or 10^{-7} M concentrations for either 1 or 2.5 min pulses. The minimum dose of AMPH required for reliable release of DA was 10^{-6} M AMPH infusion for 1 min ($T=2.7$; $p<0.026$). ANOVAs comparing the different doses of AMPH at 1 or 2.5 min pulses demonstrated a clear dose response that varied with the length of infusion ($F=5.5$; $p<0.005$ & $F=9.7$; $p<0.001$, respectively). These preliminary studies provide validation for the feasibility of using this technique to examine endogenous DA release in vitro.

290.14 DEVELOPMENT OF A SENSITIVE METHOD TO MEASURE THE ENZYMIC CONVERSION OF DIHYDROBIOPTERIN TO TETRAHYDROBIOPTERIN BY RAT BRAIN. John F. Reinhard,Jr., John Y. Chao* Gary K. Smith*and Charles A. Nichol*, Dept. Med. Biochem., The Wellcome Res. Labs. 3030 Cornwallis Rd., Research Triangle Park NC 27709

As part of a study on the availability of tetrahydrobiopterin (BH_4), the cofactor for tyrosine, tryptophan and phenylalanine hydroxylases, we have administered the BH_4 precursors L-7,8-dihydrobiopterin (BH_2) or L-6-lactyldihydrobiopterin (sepiapterin,SEP),to rats in order to elevate brain levels of BH_4. This has the advantage of elevating the natural L-erythro isomer of BH_4 since synthetic BH_4 is a mixture of of two isomers. Rat brain can form BH_4 from BH_2 using dihydrofolate reductase (DHFR)(Pollack and Kaufman J. Neurochem. 30:253,1978;Spector et al., J. Neurochem. 30:899, 1978). However, the de novo biosynthesis of BH_4 in brain and other tissues is not inhibited by methotrexate (MTX)(Nichol et al., P.N.A.S., 80:1546,1983). Since the conversion of BH_2 and SEP to BH_4 is inhibited by MTX the process is said to be DHFR-dependent. Although brain does not require DHFR for the de novo biosynthesis of BH_4 from GTP, the DHFR-dependent pathway is important as a means of elevating BH_4 by administration of BH_2 or other dihydropterins but the very low levels of DHFR may limit their conversion

Since the activity of DHFR in adult rat brain is not measurable by current spectrophotometric procedures, we have developed a liquid chromatographic assay for the enzymic conversion of BH_2 to BH_4. The method involves in vitro incubation of desalted, cell-free brain extracts with BH_2, NADPH and an NADPH regenerating system. The BH_4 formed is quantitatively converted to pterin by alkaline oxidation, is separated by liquid chromatography and detected fluorometrically (Woolf et al., J. Chrom. 274, 398, 1983). The method is linear from 100 fmol (5 x background) to 1 nmol of pterin formed. The sensitivity of this fluorometric procedure is approximately 1000 times greater than that of existing spectrophotometric assays. Enzyme activity (of desalted brain extracts) is linear both with time (to 100 minutes) and protein (from 50-620 ug). The enzyme shows an absolute requirement for NADPH, does not use NADH and is completely inhibited by 10 nM methotrexate. The enzyme's Km towards NADPH was found to be 7.5 uM, while the Km for BH_2 was determined as being 88 uM. The specific activity of the brain enzyme preparation was increased 6-fold by protamine precipitation and further increased by methotrexate affinity chromatography. Since, brain dihydrobiopterin reductase has the same properties as DHFR, this fluorometric procedure apparently can serve a sensitive assay for dihydrofolate reductase thus enabling investigation of this enzyme in brain tissues and its role in the formation of BH_4 from endogenous and exogenous precursors.

290.15 AN ISOCRATIC HPLC METHOD FOR DETERMINING PLASMA AND URINARY MHPG, USING AMPEROMETRIC DETECTION P.A. Shea and J.B. Howell*, Institute of Psychiatric Research, Department of Psychiatry, Indiana University School of Medicine, Indianapolis, IN 46223.

A rapid and simple assay for the determination of a major metabolite of norepinephrine, 3-methyoxy-4-hydroxyphenylglycol (MHPG), in both urine and blood plasma is presented. The method uses a preliminary purification of the hydrolyzed urine or non-hydrolyzed plasma sample by passing it through a short mixed bed anion-cation exchange column, allowing MHPG and other uncharged compounds to flow through, while retaining charged species. The eluate containing uncharged compounds is then extracted with ethyl acetate. The ethlyacetate extract is evaporated and the residue resuspended in the HPLC buffer (0.1M sodium acetate, pH 4.0, containing 0.1 mM EDTA). A small aliquot is injected onto a 3 µm reverse phase column and eluted at 1.5 ml/min with this buffer. Detection is by oxidative electrochemistry, at a potential of 0.9V. Total elution time is less than 25 min for both plasma and urine samples. The intraassay coefficient of variation is 9% for urine samples and 10% for plasma. Recovery of MHPG throughout the entire assay for both urine and plasma is greater than 70%. Results for MHPG determinations in 15 human subjects, both for urinary and plasma levels, yield mean values comparable to those of other procedures for determining MHPG.

290.16 DIFFUSION COEFFICIENT MEASUREMENTS OF BIOGENIC AMINE-RELATED SPECIES IN RAT STRIATUM USING IN VIVO ELECTROCHEMISTRY. M.E. Rice*, G.A. Gerhardt*, P.M. Hierl*, G. Nagy*, R.N. Adams, Dept. of Chemistry, University of Kansas, Lawrence, KS 66045.

Recently, investigators have studied the effects of the brain cell microenvironment on the diffusion characteristics of small molecules which are anionic or cationic at physiological pH (Nicholson and Phillips, J. Physiol. 296:66, 1979). These studies have been limited to non-endogenous species which are readily monitored by micro-ionselective electrodes. Using in vivo electrochemical detection, we have been able to measure the apparent diffusion coefficients (D_{App}) for several biogenic amines and related substances in rat striatum.

This technique involves the use of a miniature electrochemical electrode attached to a micro-ejection pipette. When this assembly is lowered into the striatum, the ejection pipette serves as a theoretical point source for introduction of the species of interest. After the species is ejected, electrochemical measurements monitor the spherical diffusion of the species away from the source. D_{App} for the species can be calculated using the following equation:

$$D_{App} = \frac{r^2}{6t_{max}}$$

where r is the distance between the pipette and the detector electrode and t_{max} is the time at which the maximum concentration of diffusing species reaches the electrode.

Using this method we have found that anionic species such as DOPAC and ascorbic acid diffuse at a rate approximately one-third of their solution D value. This is consistent with a D_{App} affected by the tortuosity of the extracellular diffusion path in brain tissue. Surprisingly, we find that cationic species such as DA and NE diffuse at rates which are approximately one-tenth their solution values. A compilation of these in vivo data as well as various in vitro experiments addressing the D_{App} differences between anions and cations will be presented.

290.17 4-FLUORO-3-NITROPHENYL AZIDE (FNPA), A SELECTIVE PHOTOAFFINITY LABEL FOR TYPE B MONOAMINE OXIDASE. S. Chen, J. Shih and Q.-P. Xu*. Institute for Toxicology, School of Pharmacy, Univ. of Southern California, Los Angeles, CA 90033.

Monoamine oxidase (MAO) catalyzes oxidative deamination of a number of biogenic amines. Two types of MAO catalytic activity can be separated by the use of enzyme inhibitors. Type A MAO is more sensitive to clorgyline and prefers serotonin as substrate. Type B MAO is more sensitive to deprenyl and prefers phenylethylamine (PEA) as substrate. In this report, we have investigated the molecular mechanism of two types of MAO by the use of a selective photoaffinity labeling probe-FNPA.

The effects of FNPA on type A and B MAO in rat cortex were studied using serotonin and PEA as substrates, respectively. FNPA competitively inhibited the oxidative deamination of both serotonin (Ki=3µM) and PEA (Ki=3µM). Although similar Ki values were obtained for the inhibition of the two types of MAO by FNPA, the IC_{50} values differed by more than one order of magnitude. At 100 µM serotonin, the IC_{50} for the inhibition of type A MAO by FNPA was greater than 10 µM, while the IC_{50} for inhibition of type B MAO was 0.79 µM at 10 µM PEA. The selectivity of FNPA to type B MAO was also indicated by our findings that FNPA (5µM) was able to prevent completely the deprenyl (15nM) inhibition of type B activity but it had only a weak protective effect on the clorgyline (3.5nM) inhibition of type A MAO when FNPA was co-incubated with deprenyl-(or clorgyline-) enzyme mixture.

When enzyme preparation was irradiated in the presence of FNPA, a noncompetitive inhibition of type B MAO resulted. The inhibition was apparently irreversible since there was no recovery of activity upon washing of the photolyzed FNPA-enzyme mixture. However, irradiation in the presence of PEA protected against the FNPA induced inactivation of type B MAO. The photodependent incorporation of FNPA to type B MAO was also demonstrated by the decreased labeling of the enzyme when subsequently exposed to (^{3}H)-pargyline. Under similar experimental conditions only minimal photodependent inhibition of type A MAO was found. The noncovalent attachment of FNPA to type A MAO was indicated by the competitive inhibition (Ki=3µM) of the enzyme which remained following photolysis.

The results demonstrate that FNPA is a selective photoaffinity label for type B MAO and provides strong evidence to indicate that there is a fundamental difference in the active sites of the two types of MAO (supported by NIMH grant #MH 37020).

290.18 FREE AND CONJUGATED CATECHOLAMINES AND THEIR METABOLITES IN HUMAN CEREBROSPINAL FLUID (CSF). N.S. Sharpless, L.J. Thal*, K. Tabaddor*, and L.I. Wolfson*. Albert Einstein College of Medicine, Bronx, NY 10461.

High performance liquid chromatography with electrochemical detection (LCEC) was used to measure free and conjugated dopamine (DA), norepinephrine (NE) and 3,4-dihydroxyphenylacetic acid (DOPAC) and free homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) in human ventricular and lumbar CSF and plasma. Free catechols were first adsorbed onto alumina. Then conjugated catechols in the alumina-treated CSF were hydrolyzed in $HClO_4$ at 100°C for 90 min and the released catechols were adsorbed onto alumina. Plasma was passed through small cation exchange columns and catecholamines in the column eluates were isolated as for CSF. The alumina was washed, eluted with 0.1 M oxalic acid, and 100 µl aliquots of the eluates were analyzed for NE, DA, and DOPAC by LCEC (Column: Biophase ODS-5 µm; Mobile phase: 0.1 M chloracetate, pH 3.0, 1 mM EDTA, 0.14 mM sodium octyl sulfate, and 4% methanol at 1 ml/min). The glassy carbon detector electrode was set at a potential of +0.75 V vs. Ag/AgCL. Quantitation was performed by determination of peak heights relative to the height of an internal standard (dihydroxybenzylamine) added to CSF or plasma prior to alumina or column adsorption. The limits of sensitivity for detection of the catechols in CSF were about 31 pg/ml for DA, 17 pg/ml for NE, and 32 pg/ml for DOPAC. HVA and MHPG in CSF were measured simultaneously on a µBondapak C_{18} column (Mobile phase: 0.1 M sodium acetate, pH 4.7 at 0.7 ml/min). About 20% of the DOPAC and more than 95% of the amines in CSF were conjugated. CSF levels of conjugated DOPAC were correlated with those of free HVA and DOPAC ($P<0.001$) but not with levels of conjugated DA while free MHPG levels were correlated with those of free NE ($P<0.001$) but not with levels of conjugated NE. The well established concentration gradient for acids was clearly evident for HVA and for both free and conjugated DOPAC, with highest levels in ventricular CSF and increasing concentrations in successive samples of CSF from the lumbar region. Free NE levels also increased as lumbar CSF was obtained from higher regions of the spinal cord, while there were no regional differences in CSF levels of MHPG or of conjugated NE. In contrast, levels of conjugated DA decreased as successive samples of CSF were taken from the lumbar region. Levels of conjugated catecholamines were about 10 times higher in plasma than in CSF and levels in plasma and CSF were not correlated. The results suggest that the conjugated amines in CSF and plasma are derived, at least in part, from different sources but levels of the acid hydrolyzable conjugates in CSF may not reflect central aminergic neuronal activity in adjacent CNS tissues. (Supported by NIH grant NS 09649 and by a grant from the Dystonia Medical Research Foundation.)

290.19 FACTORS AFFECTING IN VIVO ELECTROCHEMISTRY: ELECTRODE MODIFICATION BY BRAIN TISSUE AND AMPLIFICATION OF CATECHOLAMINE RESPONSES BY ASCORBIC ACID.

Curt R. Freed and Hirotoshi Echizen Depts. Med. and Pharmacol. U. Colo. Sch. Med., Denver, CO 80262

While in vivo electrochemistry has been shown to be a useful tool in neuropharmacology, controversy still persists about the identity and the concentration of compounds being detected in extracellular fluid (ECF) in brain. We have studied carbon paste electrodes to evaluate changes in electrochemical responsiveness after brain implantation. We have also examined the effect of ascorbic acid (AA) on electrochemical detection of norepinephrine (NE), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT) and 5-hydroxyindoleacetic acid(5-HIAA) in vitro. Electrodes conditioned in brain by temporary implantation (20 min) were compared with untreated electrodes for sensitivity and oxidation potential. Results:

EFFECT OF BRAIN TREATMENT ON ELECTRODE RESPONSES

	Concentration(uM)	Oxidation Pot.(volts)		Response(nA)	
		Untreated	Treated	Untreated	Treated
NE	10	0.19+0.01**	0.12+0.01	0.50+0.04**	1.27+0.15
DA	10	0.13+0.01**	0.10+0.01	0.75+0.05*	2.25+0.51
DOPAC	100	nd	0.16+0.01	nd	0.97+0.26
5-HT	10	0.29+0.01**	0.26+0.01	0.76+0.11**	3.03+0.71
5-HIAA	20	0.30+0.01	0.32+0.01	0.65+0.03	0.55+0.07
AA	1-900	nd	nd	nd	nd

*;p<0.05, **p<0.01 by t-test. nd= not detectable peak, n=5

The effect of AA on electrochemical sensitivity was examined by comparing responses with 0 and 300 uM AA (estimated ECF AA concentration). Electrode responses with 300 uM AA are expressed as ratios to responses obtained with 0 uM AA. Results:

EFFECT OF ASCORBATE ON BRAIN-TREATED ELECTRODE RESPONSES

	NE	DA	DOPAC	5-HT	5-HIAA
Conc.(uM)	1	1	100	10	20
Response Ratio	24.70	42.50	2.88	0.88	0.99

These data indicate that there is a preferential AA amplification effect for DA over DOPAC and that electrodes were 40 times more sensitive to DA than DOPAC. Therefore, it is likely that DA is the catechol species being measured in striatum in vivo despite ECF concentrations of DOPAC which are much higher than DA. The amplification effect of AA is probably related to reduction of oxidized catechols followed by reoxidation on the electrode. 5-HT and 5-HIAA do not undergo reversible oxidation in this system and are not influenced by AA. Supported by a grant from Merck Sharp & Dohme and USPHS grants RCDA HL 00782 (CRF), NS 099199 and NS 18639.

CATECHOLAMINES: ADRENERGIC PHYSIOLOGY

291.1 ANALYSIS OF ADRENALINE INDUCED HYPERPOLARIZATION IN SYMPATHETIC GANGLIA.

P.A. Smith and P.E. Rafuse* Department of Pharmacology University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

The adrenaline induced hyperpolarization (Adr_H) of *Rana pipiens* sympathetic ganglia was examined by means of the sucrose gap recording technique. The response was not antagonized by propranolol (1 μM) but was reduced to less than 60% of control by yohimbine (10 nM). Desipramine (DMI) potentiated the response, lowering the EC_{50} for adrenaline from 2.74±0.62 μM (n=5) to 0.28±0.04 μM (n=3). The hypothesis that the Adr_H might result from activation of the electrogenic Na^+ pump (Koketsu & Nakamura, Jap. J. Physiol. 26, 63, 1976) was tested by examining the effects of 10 μM ouabain, Na^+ free - Li^+ Ringer and low (0.2 mM) K^+ Ringer. To control for the effects of these substances on ionic concentration gradients, their effects on the response to acetylcholine (ACh) was also examined. High doses of ACh (10 mM) produced a biphasic response, a depolarization (ACh_D) followed by an afterhyperpolarization (ACh_{AH}). Since the ACh_D results from an increase in conductance to Na^+ and K^+, its amplitude serves as index of the action of Na^+ pump inhibitors on the Na^+ and K^+ concentration gradients. Since the ACh_{AH} results from electrogenic Na^+ pumping (Smith & Weight, Nature 267, 68, 1977) its amplitude serves as an index of the efficacy of Na^+ pump inhibitors. 2 hrs superfusion with Ringer containing 10 μM ouabain reduced the amplitude of the Adr_H to 34.7±5.6% of control (n=3). Similarly, Na^+-free, Li^+-Ringer reduced the amplitude of the response to 36.1±10.9% of control (n=6). Neither treatment appeared to have any marked effects on concentration gradients (the ACh_D). Low K^+ Ringer however enhanced the Adr_H at a time when Na^+ pumping (the ACh_{AH}) was reduced. The Adr_H was also reduced in amplitude by high (6 mM) K^+ Ringer and reversed polarity at approximately the same membrane potential as the antidromically evoked action potential afterhyperpolarization. These results are consistent with the following hypotheses; i) The Adr_H is generated by activation of α_2 adrenergic receptors. ii) A DMI sensitive inactivation mechanism for adrenaline exists in amphibian sympathetic ganglia. iii) The Adr_H is not generated by activation of electrogenic Na^+ pump. iv) The Adr_H is generated by an increase in potassium conductance which is sensitive to procedures which result in inhibition of the electrogenic Na^+ pump.

Supported by the Alberta Heritage Foundation for Medical Research and the Alberta Mental Health Advisory Council.

291.2 MULTIPLE EFFECTS OF NORADRENALINE ON LOCUS COERULEUS NEURONS IN TISSUE CULTURE.

P.G. Finlayson* and K.C. Marshall.(Spon:T.Picton) Dept. of Physiol., Univ. of Ottawa, Ottawa, Canada, K1H 8M5)

Responses to iontophoretically applied noradrenaline (NA) were studied in intracellularly recorded Locus Coeruleus (LC) neurons in explant cultures. LC neurons can be identified in the living cultures, which are prepared from newborn mice and contain tissue from the cerebellum and underlying brainstem (Dev. Neurosci. 5, 64-76, 1982).

LC neurons were studied in cultures having in vitro ages of 20-35 days. LC neurons had resting membrane potentials between -50 and -70 mV. Most LC neurons were quiescent with occasional bursts of up to 10s in duration. Other LC neurons fired at rates of 2-5 Hz with occasional short bursts. During iontophoresis of NA (0.2 M, pH 5.5) from a separate four-barrelled electrode, a few LC neurons exhibited a simple hyperpolarization. However, most LC neurons responded to NA iontophoresis by a hyperpolarization followed by a depolarization and/or an enhancement of excitatory synaptic events. The enhanced excitatory synaptic events might be explained by mechanisms such as disinhibition of non-LC neurons or an increased probability of transmitter release. To discern the direct effects of NA on LC neurons, tetrodotoxin (TTX) (100 and 500 nM) was bath perfused to block neuronal activity and thereby block synaptic transmission.

During TTX blockade of neuronal activity, an age related difference in responses was observed; i.e. monophasic 3-10 mV hyperpolarizations in LC neurons produced by NA iontophoresis were observed in cultures having in vitro ages of 26 days and older. Input resistance, determined by constant current pulses, decreased during the hyperpolarization, when membrane rectification properties are taken into account. Biphasic responses (a hyperpolarization followed by a depolarization) to NA iontophoresis during TTX blockade, occurred in most LC neurons from cultures having in vitro ages of 20-25 days. Input resistance decreased during both the hyperpolarizing and depolarizing phases of the biphasic responses. The age-related loss of depolarizations in LC neurons to NA could reflect the loss of a population of noradrenergic receptors.

(Supported by the Medical Research Council of Canada)

291.3 EFFECTS OF LOCUS COERULEUS LESIONS ON TRANSMITTER RESPONSES OF PURKINJE NEURONS IN COMBINED CEREBELLUM-BRAINSTEM EXPLANT CULTURES. K.C. Marshall and C.B. Garber*. Dept. of Physiology, Univ. of Ottawa, Ottawa, Canada K1H 8M5

Explant cultures containing tissue of the cerebellum and underlying brainstem have been shown to contain locus coeruleus (LC) neurons which can be identified in the living culture. Similar cultures of cerebellum tissue can be prepared which exclude the brainstem and LC regions. Selective lesions of LC neurons have been accomplished by incubating the combined cerebellum/LC cultures for 24 hours in feed containing 6-hydroxydopamine (500 µM) and ascorbic acid (8 mM). This treatment causes disappearance of fibers which exhibit catecholamine fluorescence and often of the LC cell bodies, but has no effect on appearance of Purkinje neurons (PN) cell bodies in the living cultures.

Responses of PN to NA have been studied using extracellular recording and iontophoresis from multibarrelled glass electrodes. NA usually inhibits spontaneous activity of PN, but frequently enhances the activity induced by iontophoretic application of L-glutamate. The responses of PN to NA and glutamate were compared in (1) cultures grown without LC, (2) in ones grown with LC and (3) in others grown with LC neurons but subjected to 6-hydroxydopamine lesions 3-5 days before the recording experiment. The responses observed were found to be qualitatively similar in the three types of culture. There appeared to be small differences in the sensitivity of PN to NA application such that those grown without LC were the least sensitive and those in 6-hydroxydopamine-treated cultures were the most sensitive. The clearest difference in PN responses was a markedly greater sensitivity to glutamate in the lesioned cultures than in the LC-containing cultures, with an intermediate sensitivity observed in those grown without LC.

The cause of the enhanced sensitivity of PN to glutamate is not known. The numbers of granule cells appear to be comparable in LC containing and LC-lesioned cultures and mean rates of spontaneous activity of PN are similar at about 5-6/sec in the two types of cultures. One possible explanation for the observed differences is that NA mechanisms can interact with glutamate receptors on PN. Such mechanisms might be suppressed in the LC-containing cultures which contain catecholamines in the bathing nutrient, and active in the LC-lesioned cultures.
(Supported by the Medical Research Council of Canada)

291.4 ADRENERGIC TRANSMISSION IN HIPPOCAMPUS-LOCUS COERULEUS DOUBLE GRAFTS IN OCULO: DEMONSTRATION BY IN VIVO ELECTROCHEMICAL DETECTION. L. Olson*, G.A. Gerhardt, M.R. Palmer, Å. Seiger*, R.N. Adams and B.J. Hoffer*. Dept. of Chemistry, Univ. of Kansas, Lawrence, Kanses 66045; Dept. of Pharmacology, Univ. of Colorado Health Sciences Center, Denver, Colo., 80262; Dept. of Histology, Karolinska Institute, Stockholm, Sweden.

In vivo electrochemical detection was used to study transmitter release from a synaptically or pharmacologically stimulated noradrenergic isolated pathway formed by double in oculo brain tissue grafts. Retinal illumination, which activates cholinergic nerve fibers that grow into intraocular grafts from the autonomic ground plexus of the iris, produced increases in the concentration of electroactive species in the hippocampal portion of intraocular locus coeruleus-hippocampus double grafts. This response was potentiated after cholinesterase inhibition, and mimicked by perfusion of carbachol, a muscarinic agonist. Much smaller increases in the electroactive species were measured in reserpinized animals, and this minimal response was completely eliminated by the subsequent inhibition of catecholamine synthesis with alpha-methyl-p-tyrosine. Taken together, the data suggest that synaptically released transmitter in these isolated brain circuits can be measured using in vivo electrochemical detection techniques, and lend further support to the hypothesis that in oculo locus coeruleus grafts develop a functional catecholaminergic input to sequentially grafted hippocampus.
(Supported by NSF Grant # BNS82-09608, by USPHS Grant ES-02011, and by Swedish Medical Research Council Grants 14P-5864, 14X-03185, 14P-0665 and 25X-06326, Magnus Stiftelse, Karolinska Institute Fonder.)

291.5 HALOPERIDOL ALTERS ACTIVITY OF LOCUS COERULEUS NEURONS. T. Dinan*, R. Shaver* and G. Aston-Jones (Spon: N. E. Spear). Center for Neurobehavioral Sciences, SUNY, Binghamton, NY 13901.

The therapeutic efficacy of neuroleptics in the treatment of psychotic disorders is well established, as is their tendency to produce side effects. Animal studies of such side effects have in recent years tended to focus on nigro-striatal dysfunctioning, as manifested by extra-pyramidal symptomatology. It has been suggested that post-psychotic depression also represents a neuroleptic-induced disorder, at least in some cases. We therefore examined the effects of haloperidol (Hal) on the physiology of norepinephrine (NE)-containing locus coeruleus (LC) neurons, as these cells have been implicated in depressive disorders.

Extracellular recordings were obtained from single NE-LC neurons in adult male albino rats. Chloral hydrate was continuously administered intraperitoneally throughout experiments with a syringe pump to maintain a relatively constant level of anaesthesia. All recordings were obtained from histologically verified NE-LC neurons. For one cell in each animal ten minutes of baseline activity was recorded before Hal was administered intraperitoneally (0.5mg./kg). Spontaneous discharge rate was then monitored for as long as the cell could be held (30-60 min). The mean rate of baseline discharge was 1.1 ± 0.1 Hz. Hal administration increased discharge in each of 11 cells examined, with mean rates becoming significantly faster by 30 min post-drug (mean rate = 2.2 ± 0.1 Hz). Following this analysis for one cell in each animal, additional neurons were recorded up to 2 hr after Hal administration. Analysis of these data revealed that the effect of Hal to increase NE-LC activity reached a plateau by 1 hr (mean rate = 3.2 ± 0.2 Hz) which remained stable for at least another hr. Further experiments are in progess to examine the effects of Hal on sensory responsiveness and post-excitation inhibition. Results of preliminary studies with chronically administered Hal reveal decreased NE-LC firing, with many cells being non-spontaneous.

This work was supported by NINCD Grant NS 19360 and BRSG Grant SO7RR7149-09 from NIH.

291.6 NOREPINEPHRINE-INDUCED HYPERPOLARIZATIONS AND DEPOLARIZATIONS IN PARASYMPATHETIC NEURONS ARE MEDIATED THROUGH ALPHA$_2$ AND ALPHA$_1$ ADRENOCEPTORS, RESPECTIVELY. P. Shinnick-Gallagher, T. Nakamura*, M. Yoshimura*, and J. Gallagher. Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX.

A norepinephrine (NE)-induced membrane hyperpolarization, depolarization or biphasic response was recorded intracellularly in neurons of cat vesical pelvic ganglia when applied in a drop, or by continuous superfusion, iontophoresis or pressure ejection. At lower concentrations, NE (.01 - 1µM) produced only a hyperpolarization whereas biphasic responses and membrane depolarizations were recorded at higher concentrations (10µM). Propranolol (1µM) had no effect on the NE responses, whereas phentolamine (1µM) blocked all types of NE responses. Furthermore, yohimbine (1µM) an alpha$_2$ adrenoceptor antagonist selectively blocked the NE hyperpolarization and prazocin (10^{-7}M) a alpha$_1$ adrenoceptor antagonist blocked NE depolarizations. Alpha$_1$ agonists, phenylephrine and methoxamine, also induced membrane depolarizations.

The NE-induced hyperpolarization was occasionally accompanied by a conductance increase while the NE depolarization was routinely associated with a conductance decrease. The NE-induced hyperpolarization had a null potential of -100mV, and was dependent on K^+ concentration. The calcium antagonist, Cd, reversibly blocked the hyperpolarizing NE response and converted biphasic NE responses into pure depolarizations. Intracellular injection of EGTA (250 pulses of 1.5 nA, 200 msec @1Hz) produced a time dependent depression of the NE induced hyperpolarization with no consistent change in membrane conductance. Biphasic NE responses were also converted to depolarizations after EGTA injection. The NE depolarization reversed in polarity around the potassium equilibrium potential.

The results suggest that the NE-induced hyperpolarization is due to direct activation of a calcium dependent, K^+ conductance and is mediated through an alpha$_2$ adrenoceptor, and that the NE depolarization may be due to closure of K^+ channels and is mediated by alpha$_1$ adrenoceptors.

We believe this is the first electrophysiological evidence for the presence of both on α_1 and α_2 adrenoceptor on the same neuronal postsynaptic membrane. Supported by NIH NS16228.

291.7 HYPERPOLARIZATION OF PRIMARY AFFERENT TERMINALS MEDIATED BY α_2-ADRENOCEPTORS. C.J. Wohlberg, J.C. Hackman, G.P. Ryan*, and R.A. Davidoff. Neurophysiology Laboratory, VA Medical Center, and Depts. of Neurology and Pharmacology, Univ. of Miami School of Medicine, Miami, FL 33101.

Although epinephrine (E) and norepinephrine (NE) and adrenoceptors are known to be present in the spinal cord, the mechanism by which these catecholamines (CAs) function is not known. The present experiments were designed to characterize receptors in the spinal cord activated by CAs.

We used the isolated, hemisected frog spinal cord superfused with HCO_3^--buffered Ringers at 15°C. Responses were recorded from DRs using sucrose gap techniques. E and NE (0.01-100 μM, 10-120 sec applications) hyperpolarized primary afferent terminals (0.3-1.5 mV) for long durations (up to 6 min) and reduced the frequency of spontaneous DR activity. Both responses seemed to depend on intact synaptic activity, since they were diminished by agents known to reduce synaptic transmission - Mn^{++} (1.5 mM), tetrodotoxin (1.25 μM) and mephenesin (1.0 mM).

The responses elicited by these CAs appear to be caused by activation of α receptors since both the hyperpolarization and the reduction in background activity were inhibited by phentolamine (10 μM, 60 min), a non-selective α-adrenoceptor antagonist. Furthermore, α_2 receptors seem responsible since yohimbine (0.1-10 μM, 30 min) or piperoxane (1-10 μM, 30 min), both selective α_2-antagonists were potent blockers of CA effects. The selective α_1-antagonists prazosin (10 μM, 60 min) and corynanthine (1 μM, 60 min) were ineffective as were the β-antagonists sotalol (1-10 μM, 60 min) and propranolol (1-10 μM, 60 min).

These data were supported by the finding that the actions of E and NE were mimicked by α-methyl-norepinephrine, an α_2-selective agonist, but not by phenylephrine (1-100 μM, 30 sec) and methoxamine (1-100 μM, 30 sec), both α_1-adrenoceptor agonists.

Application of various MAO inhibitors (pargyline, iproniazid, isoproxazide and tranylcypromine; 1-10 μM, 60 min) failed to potentiate the hyperpolarizations. U-0521, a COMT blocker, potentiated the response elicited by E but not that of NE. Imipramine, a CA reuptake blocker, significantly increased the duration of both the E and NE hyperpolarizations.

These results suggest that adrenoceptors of the α_2-subtype are responsible for mediation of the effects produced by E and NE in the spinal cord and recorded from primary afferent terminals. It is possible that these CAs play a significant role in the modulation of sensory input into the spinal cord. Supported by VAMC funds (MRIS 1769), UPHS Grants NS 17577 and HL 07188 and a grant from the National Parkinson Foundation, Inc.

291.8 COCAINE: CORRELATION BETWEEN ELECTROPHYSIOLOGICAL AND BIOCHEMICAL EFFECTS IN RAT HIPPOCAMPUS IN VITRO. Robert P. Yasuda*, Nancy R. Zahniser and Thomas V. Dunwiddie. Dept. Pharmacology, Univ. Colo. Health Sciences Center, Denver, CO 80262.

The ability of cocaine to block high affinity uptake of norepinephrine (NE) has been well characterized in the peripheral and the central nervous systems. However, the electrophysiological actions of cocaine in the central nervous system remain largely unknown. In the present experiments, we confirmed the ability of cocaine to reduce the sodium- and temperature-dependent uptake of ^{3}H-NE in slices of rat hippocampus. At low doses of cocaine (2.5 μM) this high affinity uptake was reduced by 30%. The tricyclic antidepressant desipramine had a similar effect.

The effects of perfused cocaine on evoked potentials in the CA1 region of the in vitro hippocampal slice were subsequently examined under conditions identical to those used in the uptake studies. High concentrations of cocaine (10-500 μM) elicited decreases in antidromically and synaptically evoked potentials consistent with local anesthetic actions on neuronal membranes; other local anesthetics (procaine, lidocaine) produced similar changes in these responses. Lower concentrations of cocaine (<10 μM) produced no direct effects on the observed population spike amplitude, but did affect subsequent responsiveness of brain slices to co-perfusion with low concentrations of NE. Perfusion with a concentration of NE (0.5 μM), which alone did not significantly affect the population spike amplitude (mean increase in amplitude = 14 ± 14%) produced large increases in the presence of 2.5 μM cocaine (93 ± 22%). However, cocaine did not affect the maximal response elicited by NE. Similar potentiations were seen with imipramine and desipramine.

Using the in vitro rat hippocampus, it is possible to differentiate two distinct pharmacological responses to cocaine. First, high concentrations elicit what appear to be direct local anesthetic effects on hippocampal neurons. The ability of lower doses of cocaine to augment the changes in population spike amplitude elicited by NE appears to be a second, more specific effect which reflects the ability of cocaine to block the uptake of NE into adrenergic nerve terminals in the rat hippocampus.

This work was supported by GM-07635 (R.P.Y.), NS-09199 (N.R.Z.) and VA-394463116-01 and DA-02702 (T.V.D.).

291.9 IN VITRO SUPERFUSION OF NOREPINEPHRINE POTENTIATES THE PERFORANT PATH EVOKED FIELD POTENTIAL IN THE DENTATE GYRUS. J-C. Lacaille and C.W. Harley. Department of Psychology, Memorial University of Nfld., St. John's, Nfld. A1B 3X9.

The effects of norepinephrine (NE) superfusion on the electrophysiological response of the granule cells of the dentate gyrus were assessed in the hippocampal slice preparation.

Slices (300-400 μm thick) were obtained from rat hippocampi and were maintained submerged in oxygenated artificial CSF. Granule cells were stimulated using bipolar tungsten microelectrodes positioned in the perforant path and evoked field potentials were recorded at the granule cell body layer. Field potentials were digitized by a microcomputer for on-line analysis of EPSP amplitude and population spike onset latency and amplitude. For drug applications the slices were superfused for 10 min with CSF containing 1, 5, 10, 25 or 50 μM NE.

Our results indicate that superfusion with NE increases population spike amplitude but does not affect EPSP amplitude or population spike onset latency. The NE-induced potentiation was dose-dependent, with 10 μM NE being the most effective. During the 10th minute of superfusion, 10 μM NE produced a mean increase in population spike amplitude of 18% (range 0-36%), whereas 25 μM NE produced a 13.6% increase (range 3-34%) and 50 μM 9.6% (range 0-23%). However, 1 and 5 μM NE did not produce any changes. For some slices we have followed the potentiation produced by 10 μM NE for longer periods. In many slices we have observed that the population spike amplitude remained above pre-NE levels 30 min following NE application. To ascertain whether NE-induced potentiation was mediated via a beta receptor, we applied NE together with timolol (TIM), a non-selective beta antagonist with minimal anesthetic-like properties. Five μM TIM blocked the potentiation produced by 10 μM NE. TIM alone did not affect the evoked field potential.

These results indicate that NE can increase the excitability of the dentate granule cells in vitro via a beta receptor and that this increased excitability can be long-lasting.

(Supported in part by NSERC A-9791 to C.W.H.)

291.10 INTERACTIONS OF NOREPINEPHRINE AND SEROTONIN WITH VISUALLY EVOKED RESPONSES OF SIMPLE AND COMPLEX CELLS IN AREA 17 OF RAT CORTEX. B.D. Waterhouse, S.A. Azizi, R.A. Burne and D.J. Woodward, Dept. Cell Biology, U.T. Health Sci. Ctr., Dallas, TX 75235

Previous investigations carried out in our laboratory have demonstrated that norepinephrine (NE) can enhance excitatory and inhibitory responses of somatosensory cortical neurons evoked by punctate, mechanical stimulation of the forepaw. In contrast, serotonin (5HT) suppresses the responsiveness of somatosensory cortical neurons to the same type of forepaw stimulation. The present study was conducted to examine the actions of NE and 5HT on visually evoked responses and receptive field (RF) properties of simple and complex cells recorded from the visual cortex (area 17) of halothane anesthetized rats. Visual responses of 15 simple and 20 complex cells, evoked by computer controlled presentation of moving visual stimuli, were examined before, during and after low level microiontophoretic application of NE (1-20nA) or 5HT (1-30nA). Drug induced changes in stimulus evoked and spontaneous discharge were quantitatively assessed by computer analysis of perievent histograms.

In 5 complex and 4 simple cells, iontophoretic administration of NE produced an absolute increase in evoked spiking while suppressing or having no effect on spontaneous discharge. In 7 additional cases (3 complex, 4 simple), NE exerted a differential depressant action on evoked and spontaneous discharge such that stimulus evoked excitation was suppressed less than background firing yielding a relative enhancement of the excitatory response. Stimulus bound inhibitions were also augmented in 3 of 3 complex and 4 of 5 simple cells. In one case, a subthreshold inhibition was revealed during administration of NE at a level which caused no change in background firing. The facilitating action of NE on evoked excitation was mimicked by phenylephrine but not isoproterenol; whereas isoproterenol was capable of producing an NE-like potentiation of stimulus bound inhibitions.

In contrast to these noradrenergic actions, 5HT reduced the efficacy of stimulus induced excitation and inhibition in 8 of 11 and 2 of 2 cells, respectively. In some cases, the net effect of NE or 5HT induced changes in excitatory or inhibitory components of compound visual responses was to alter the dimensions of RF's or bias the direction selectivity of single neurons. For example, NE sharpened RF borders in 9 cells, whereas 5HT increased the width of RF's in 3 cases.

In summary, these results suggest that NE and 5HT may modulate by complimentary actions the transfer of specific sensory information within the visual cortical circuitry. (Supported by NINCDS NS-18081 and the Klingenstein Foundation to BDW and NIDA DA-02338 and the Biological Humanics Foundation to DJW).

291.11 ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL CHARACTERISTICS OF NEURONS IN THE PREFRONTAL CORTEX OF THE RAT, L.A. Chiodo and B.S. Bunney. Dept. Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06510.

Previous work from our laboratory has demonstrated that neurons located in the prefrontal cortex of the rat are preferentially sensitive to microcoiontophoretically applied norepinephrine (NE) or dopamine (DA) depending upon their anatomical location (NE sensitive cells = layers II & III; DA sensitive cells = layers V & VI; Bunney & Aghajanian, Life Sci., 19: 1783, 1977). In the present study we used in vivo single-unit extracellular recording and microiontophoretic techniques to further characterize the electrophysiological and pharmacological nature of these prefrontal cortex (PFC) neurons in the anesthetized rat.

As previously reported PFC cells which were preferentially inhibited by the iontophoretic application of NE (>65% inhibition with ejection currents of 20-30 nA, applied for 1 min) were located in layers II & III. These cells displayed biphasic (-/+) action potentials, spike durations of 2.0 ± 0.1 (mean ± S.E.M.) msec and peak-to-peak amplitudes of between 0.2 and 1.0 mV. Spontaneous discharge was irregular (mean ± S.E.M. = 3.2 ± 0.6 spikes/second) with 32% of the action potentials occurring in bursts typically containing 2-6 spikes. PFC neurons preferentially sensitive to DA iontophoresis (>65% inhibition with ejection currents of 20-30 nA, applied for 1 min) were again found predominantly in layers V & VI. The extracellular electrical characteristics of the cells were similar to those observed for the NE-sensitive neurons. Thus, DA-sensitive cells discharged irregularly with a mean firing rate of 3.3 ± 0.5 spikes/sec. Action potentials were biphasic (-/+) action potential with durations of 1.8 ± 0.1 msec and amplitudes of 0.2-1.0 mV. Approximately 27% of their action potentials occurred in bursts of 2-6 spikes. The average interspike interval between action potentials occurring in a burst for PFC neurons was 9.8 ± 0.5 msec.

The DA-induced inhibition of PFC neurons in layers V & VI appeared to be a specific effect. Thus, in these neurons NE iontophoresis (20-30 nA, 1 min) was only 20% as potent as DA. Moreover, iontophoretic application of either the alpha$_2$-NE agonist clonidine or beta-NE agonist isoproterenol had no effect. In addition, while the co-iontophoresis of the DA antagonist trifluoperazine readily blocked the inhibitory effects of DA, the alpha-NE antagonists piperoxane and prazosin and the beta-NE antagonist sotalol had no effect. Thus, as is true of DA somatodendritic autoreceptors, DA receptors on neurons in the deep layers of the PFC appear to be neither alpha nor beta, but uniquely DA. (Supported by USPHS grants MH-28849, MH-25642, NRSA Fellowship MH-08848 and the State of CT.)

291.12 NALOXONE REVERSES HYPOTHALAMIC CATECHOLAMINE ALTERATIONS CAUSED BY ACUTE DEHYDRATION. Harry A. Klemfuss* and Lewis S. Seiden. Dept. Pharmacological & Physiological Sciences, The University of Chicago, Chicago, IL 60637.

Naloxone injection decreases the volume of water ingested following intracellular dehydration with hypertonic saline (HTS) injection or extracellular dehydration with polyethylene glycol (PEG) injection. We have previously found that HTS treatment increases metabolism of norepinephrine in the paraventricular nucleus of the hypothalamus (PVN) but not in any other hypothalamic area. Dopamine metabolism in the medial hypothalamus is also sensitive to changes in plasma osmolarity. Other procedures causing equal or greater water intake, including PEG, have no effect on catecholamine metabolism in the PVN or medial hypothalamus (Klemfuss & Seiden, submitted). This study examined the effect of naloxone pretreatment on the metabolism of hypothalamic catecholamines following HTS and PEG treatment in the rat. Injection parameters; Saline or 10 mg/kg naloxone HCl sc. at 120, 60, and 30 min before death; saline or 200 mg/kg alpha-methyl-p-tyrosine methyl bromide (AMT) 60 min before death; 0.15 M or 1 M (HTS) NaCl (15 ml/kg) 105 min before death; or 40% PEG (15 ml/kg) 180 min before death. Catecholamine concentration was measured by high pressure liquid chromatography with electrochemical detection in PVN, preoptic area, lateral hypothalamus, anterior hypothalamus, and medial hypothalamus. Metabolism rate was determined by the difference between catecholamine levels in AMT and Saline-treated rats. As expected, HTS significantly increased norepinephrine metabolism in the PVN and decreased medial hypothalamic dopamine metabolism. Both effects were reversed by naloxone. No other treatment or drug effect was statistically significant except that norepinephrine metabolism in the entire hypothalamus was increased by naloxone treatment ($p < .05$). Naloxone reversal of the alterations in the hypothalamic catecholamine metabolism caused by HTS may be related to naloxone's inhibition of water ingestion. (Supported by USPHS MH-11191; L.S.S.: RSA MH-10562; MH-14274, Training Grant).

291.13 IN VIVO ELECTROCHEMICAL DETECTION OF THE EFFECTS OF CO_2 ON BIOGENIC AMINE RELEASE. A. Oke, T. Kent*, S. Preskorn, R.N. Adams. Dept. of Chem., University of Kansas, Lawrence, KS 66045 (AO and RNA) and Dept. of Psychiatry, Univ. of Kansas Medical Center, Kansas City, KS 66103 (TK and SP).

In vivo electrochemistry measures the release of oxidizable substances from brain. A new nafion-treated electrode has been developed which excludes anionic compounds such as amine acid metabolites, uric acid and ascorbic acid (Gerhardt et al., subm.). Garcia de Yebenes Prous et al. (Naunyn-Schmiedeb. Arch. Pharmacol., 1978) suggested that CO_2 increases the release of NE and 5-HT while decreasing the release of DA as analyzed in post-mortem tissue. In addition, Elam et al. (Brain Res., 1981) and Levine et al. (J. CBF&Met., 1981) showed that CO_2 increases the firing rate of the locus coeruleus. Our preliminary findings are consistent with these results.

Rodents (N=15) were anesthetized (chloral hydrate, 400 mg/kg i.p.), intubated and passively ventilated with N_2 (55-70%), O_2 (30%) and CO_2 (0-15%). Graphpoxy electrodes with and without nafion coating (ca. 150 µm) were employed throughout the study. Serial 1 sec chronoamperometric readings were obtained at 15 sec intervals using an applied potential of 0.55 V. Readings were obtained with varying amounts of CO_2 in cortex, hippocampus and thalamus. Fifteen percent CO_2 consistently produced a 2-5 µM increase in transmitter concentration. Acute radiofrequency locus coeruleus lesions produced a 30-80% reduction in this signal. Chronic locus coeruleus lesions (2 weeks old) produced less consistent results, which may indicate adaptation of the system. Interestingly, lower doses of CO_2 at times produced a sawtooth-like signal with small increases and decreases around the baseline. Conversely, the caudate demonstrated a dose-dependent decrease in the signal.

These results address several points: (a) in vivo chronoamperometry is capable of measuring rapid on-going aminergic alterations which correspond to known post-mortem and electrophysiological data; (b) this methodology appears to be capable of measuring subtle alterations in release and uptake of neurotransmitters, as suggested by the biphasic responses obtained with low-dose CO_2; (c) peripheral artifacts due to possible damage to blood-brain barrier appear to be minimal, since CO_2 did not cause a consistent increase in the concentration of electroactive species measured as would be expected with a damaged blood-brain barrier and global increase in cerebral blood flow due to CO_2.

291.14 PHENOXYBENZAMINE BUT NOT HALOPERIDOL REINSTATES ALL MOTOR AND SENSORY DEFICITS IN CATS FULLY RECOVERED FROM SENSORIMOTOR CORTEX ABLATIONS. David A. Hovda, Dennis M. Feeney, Ann A. Salo*, and Michael G. Boyeson, Departments of Psychology and Physiology, University of New Mexico, Albuquerque, NM (87131)

Our recent work has focused on the ability to accelerate recovery of function after brain injury by administering amphetamine. We have proposed that this AMP induced acceleration of recovery is due to its effects on the catecholamines (CA) (Feeney, et al., Science, 217, 815-817, 1982; Hovda & Feeney, Neuroscience Abstracts, 8, 358, 1982). We now present evidence that a functioning noradrenergic (NE) system is necessary for sustaining recovery of function after sensorimotor cortex injury in the cat. After unilateral sensorimotor cortex lesions, cats display a contralateral hemiparesis, extensor rigidity, limb hyperextension, and a somatosensory loss. These deficits recover by 10 days after surgery, and a deficit which persists for at least 60 days in locomotor ability is seen when the cats are placed on a narrow elevated beam. In 9 cats fully recovered from injury (3-8 mos postsurgery) a single administration of phenoxybenzamine (PBZ, 5 mg/kg, i.p.) produced a weak reinstatement of all the contralateral deficits. When given 10 mg/kg of PBZ the reinstated deficits were severe and began 3 h postinjection and persisted for up to 4 days. This dose of PBZ had no effect on normal cats except to make them drowsy. A dose of 10 mg/kg of pentobarbital produced only some hyperextension of the affected limbs in injured animals but also produced this same effect in normal uninjured controls. Therefore, the PBZ effect is not due to a soporific effect. A dose of .8 mg/kg of haloperidol produced only some hyperextension of the affected limbs in one animal and had no effect on any other behavioral measures. This lack of effect of haloperidol, even at this high dose, confirms our previous observations that it only retards recovery when given early after injury. The PBZ results are similar to the report of a worsening of symptoms in stroke patients given PBZ at doses which are used to reduce hypertension (Meyer, et al., Stroke, 7, 158-167, 1976). Therefore PBZ is contraindicated in cases of brain injury. We conclude that NE functioning is required for maintaining behavioral recovery after brain injury. Supported by NIH MBRS grant RR08139-07 and a grant from the Pennwalt Pharmaceutical Corporation.

291.15 EFFECTS OF YOHIMBINE AND PRAZOSIN ON THE NATRIURETIC AND KALIURETIC RESPONSES TO NORADRENALINE. W.A.Saad, S.Sedenho*, J.V. Menani*, William A.Saad*, L.A.A.Camargo* and A.Renzi*. Deptº de Fisiologia e Patologia, FOA-UNESP, Araraquara, SP e Deptº Cirurgia, FMSP-USP, São Paulo.

It has been demonstrated that there is participation of CNS in the regulation of the hidromineral equilibrium. It was also established that structures such as the paraventricular nucleus, lateral preoptic area, septal area, posterior, medial, and lateral hypothalamus are implicated in the regulation of the urinary excretion of Na^+ and K^+. Thus the α adrenergic system when stimulated, increases the excretion of these two cations and the β adrenergics acts as a reducer. The pharmacological and functional differences in possynaptic and presynaptic α-adrenoceptors led to the designation α_1 and α_2 respectively. It has been recommended that the classification α_1 and α_2 should be used independent of the location and function of α-adrenoceptors, according to its affinity to agonists and antagonists. The objective of this communication was to verify if one or more than one type of α-adrenoceptor of the rat medial hypothalamus (MH) was being envolved in the urinary excretion of Na^+ and K^+. Male rats weinghing 250-300 g, carrying stainless steel cannulae, in the MH, were used in the experiment. First a dose-response curve was stablished with noradrenaline (a non-selective α_1 and α_2-adrenoceptor agonist) used in doses of 10, 20, 40, 80, 160 and 320 nM. The previous administration of 10 and 40 nM of yohimbine (a selective α_2-adrenoceptor antagonist) in others animals, shifted the noradrenaline log dose-response curve to the left. On the other hand, the prazosin (a selective α_1-adrenoceptor antagonist) in doses of 10 and 40 nM shifted the noradrenaline log dose-response curve to the right, which indicated that the 40 nM dose of prazosin inhibited almost all the noradrenaline natriuretic and kaliuretic response. This data suggests that the α_1-adrenoceptors of the rats MH would be preferencially envolved in the increase of Na^+ and K^+ excretion.

Supported by FAPESP grants 81/093-8.

291.16 NORADRENERGIC ACTIVITY AND (Na^+,K^+)-ATPase: EFFECTS OF NOREPINEPHRINE DEPLETION AND CALORIC INTAKE ON (Na^+,K^+)-ATPase AND NORADRENERGIC PARAMETERS IN BRAIN AND PERIPHERAL TISSUES. Alan C. Swann, Department of Psychiatry, University of Texas Medical School, Houston, TX 77025.

We have previously shown that noradrenergic stimulation increased brain (Na^+,K^+)-ATPase in vivo and that this stimulation required binding to noradrenergic receptors (Brain Res 260:338). 6-hydroxydopamine lesions of the dorsal noradrenergic bundle also reduced cerebral cortex (Na^+,K^+)-ATPase (J Neurochem 38:838). These results raise questions as to the reversibility of the effects of noradrenergic changes on (Na^+,K^+)-ATPase, the role of norepinephrine-regulated (Na^+,K^+)-ATPase in physiologic situations where (Na^+,K^+)-ATPase activity is increased, and the generality of noradrenergic regulation of (Na^+,K^+)-ATPase in the sympathetic nervous system. This report examines the time course of (Na^+,K^+)-ATPase regulation in brain and heart using the noradrenergic neurotoxin DSP4, which has the advantages of being selective for norepinephrine over dopamine, crossing the blood-brain barrier, and having reversible effects on norepinephrine in peripheral tissues. For a possible physiologic role of increased (Na^+,K^+)-ATPase, we examined the effect of norepinephrine depletion by 6-hydroxydopamine on the increase in brown adipose tissue (Na^+,K^+)-ATPase after sucrose overfeeding, which is thought to be a mechanism of dietary thermogenesis. In cerebral cortex, ouabain binding and phosphatase activity associated with (Na^+,K^+)-ATPase were reduced by 30% 16 hours after DSP4 (50 mg/kg ip), decreased further after seven and fourteen days, and remained decreased five weeks after the single DSP4 injection. The time courses of decreases in desipramine binding and norepinephrine content were similar. There were no changes in dopamine content. In heart, (Na^+,K^+)-ATPase and noradrenergic parameters were decreased 16 hours and seven days after DSP4 but had returned to control levels by 14 days. In both brain and heart, there were significant correlations between ouabain binding and norepinephrine content. Sucrose feeding increased ouabain binding and phosphatase activity in brown adipose tissue. This increase was prevented by parenteral 6-hydroxydopamine, without effects on caloric intake. These data suggest that (Na^+,K^+)-ATPase is regulated by norepinephrine in both brain and sympathetic nervous system. In peripheral tissues, reappearance of norepinephrine after lesions is associated with reversal of the reduction in (Na^+,K^+)-ATPase. Stimulation of (Na^+,K^+)-ATPase by increased caloric consumption may provide a model for physiologic regulation of (Na^+,K^+)-ATPase by norepinephrine.

Supported in part by USPHS Grant MH37141, Research Development Award MH00415, and the Texas Affiliate of the AHA.

291.17 KINETIC PROPERTIES OF TYPE A AND B MONOAMINE OXIDASE AND THE METABOLISM OF STRIATAL DOPAMINE. A. J. Azzaro, G. S. Carter*, J. Frost*, J. Liccione*, J. Kotzuk*, J. King*, D. D. Schoepp*, and S. Schochet*. Departments of Neurology, Behavioral Medicine/Psychiatry, Pharmacology/Toxicology, and Pathology, West Virginia University Medical Center, Morgantown, WV 26506.

Differences exist among mammalian species for the ratio of brain type A & B monoamine oxidase (MAO). Rodents, for example, demonstrate a preponderance of type A MAO while primates possess mostly type B MAO. In the present study, kinetic properties were determined for dopamine (DA) deamination by MAO in the striatum of three mammalian species which contain different ratios of type A & B MAO. An attempt was made to determine the importance of MAO isozyme ratio and kinetics to the endogenous metabolism of striatal DA.

Reaction rates for DA metabolism by type A & B MAO were determined following the preincubation of striatal mitochondrial fractions with selective concentrations of clorgyline (type A inhibitor) or deprenyl (type B inhibitor). The concentration of (^{14}C)DA was varied from 0.025mM to 0.5mM. The study was conducted in tissue from rats, guinea pigs and autopsied humans. With saturating concentrations of (^{14}C)DA, the MAO ratio (% type A/% type B) was found to be 76/24 for rat, 28/72 for guinea pig and 25/75 for autopsied human. Affinity determinations showed that type A MAO has a 2.5 fold higher affinity for DA in rat striatum (K_{m_a} = 0.12mM; K_{m_b} = 0.30mM). By contrast, equal affinities for DA by type A & B MAO was observed in guinea pig striatum and autopsied human caudate nucleus (guinea pig: K_{m_a} = 0.39mM, K_{m_b} = 0.40mM; human: K_{m_a} = 0.19mM, K_{m_b} = 0.13mM).

The metabolism of endogenous striatal DA was determined in rat and guinea pig following a 2 hour pretreatment with selective doses of clorgyline or deprenyl. DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) were determined. In rat striatum, clorgyline completely inhibited DOPAC and HVA formation and caused a 4 fold increase in 3-MT. Deprenyl was without effect. By contrast, either clorgyline or deprenyl were partially effective in altering guinea pig striatal DA metabolism and complete inhibition of DA deamination required administration of both agents.

The results suggest that the affinity for substrate, and not the isozyme ratio, is the primary factor in the deamination of DA by type A & B forms of MAO. Therefore, guinea pig striatum may serve as a good animal model system to study human DA metabolism. (Supported by WVU Medical Corporation)

291.18 BIOCHEMICAL RESPONSE OF DOPAMINE NEURONS TO AYTPICAL NEUROLEPTICS IS NOT DEPENDENT ON AUTORECEPTORS. Matthew P. Galloway* & Robert H. Roth, (SPON:Z.Smith), Pharmacology & Psychiatry, Yale University School of Medicine, New Haven, CT. 06510

Dopamine (DA) neurons projecting to the prefrontal (PFC)and cingulate cortex have recently been shown to be devoid of autoreceptors, in contrast to those DA-neurons projecting to the piriform cortex or caudate. These conclusions are based on biochemical evidence (eg. metabolite responses to DA antagonists and agonists, Bannon et al., 1983) and electrophysiological data (eg. firing rates after agonist administration, Chiodo et al,1983). Typical neuroleptics such as haloperidol (hal) and fluphenazine (flu) exert a profound effect on DA neurons with autoreceptors, namely they elevate DA synthesis, turnover, and levels of the metabolites DOPAC and HVA. Paradoxically, atypical neuroleptics are much less effective in changing DA metabolism. Also, chronic treatment with haloperidol produces a state of "depolarization inactivation" in the A9 & A10 DA cell bodies, but not in those innervating the PFC. Since these effects are thought to result from the antagonism of autoreceptors, the diminshed response to these agents in the PFC is consistent with the lack of terminal autoreceptors on these neurons. Relying on the differential response of DA neurons to typical neuroleptics, we measured DA metabolism in the aforementioned DA projections after atypical neuroleptics in an attempt to determine the importance of autoreceptors in the biochemical response to these compounds.

By using HPLC-EC or GC-MS to measure levels of DA, DOPAC, and HVA, we found that acute hal or flu administration caused a 3-4 fold increase in metabolites in the caudate, n. accumbens, and piriform cortex while in the PFC and cingulate cortex the increase was about 0.8 fold. Administration of clozapine (20 mg/kg) or sulpiride (30mg/kg) produced a modest but uniform increase in DOPAC(50-100%) in all tissues studied. This observation indicates that atypical neuroleptics such as clozapine and sulpiride stimulate DA metabolism through a mechanism independent of autoreceptors, possibly through activation of feedback loops.

We have also been interested in the biochemical correlates of the electrophysiological quiesence noted in DA neurons after chronic hal. After chronic hal administration (0.5 mg/kg/day, p.o.), several paramaters of terminal DA function were determined. It was found that tyrosine hydroxylation in vivo (after NSD) in the caudate or PFC was unchanged by hal treatment. Also, turnover of DA (after α-MT) or GBL-stimulated DA synthesis was unaltered in any region by this treatment. There was a consistent 30-40% decrease in DOPAC levels in all areas studied however DA levels were unchanged. Cerebellar NE was elevated (30%), however the synthesis and turnover of NE were equal to control. (Supported by MH-14092 and DA-05209 and the State of Conn.)

291.19 APOMORPHINE SELECTIVELY REVERSES THE IMPULSE INDUCED INCREASE IN DOPAMINE SYNTHESIS IN MESOCORTICAL DOPAMINE NEURONS WITH AUTORECEPTORS. See-Ying Tam*, Michael J. Bannon, Robert H. Roth. Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

Recent biochemical and electrophysiological studies have demonstrated that the mesocortical dopamine (DA) neurons projecting to the prefrontal and cingulate cortices are devoid of autoreceptors, whereas those projecting to the piriform cortex possess nerve terminal and cell body autoreceptors (Bannon et al., Eur. J. Pharmacol., in press; Chiodo et al., J. Neurosci., in press). Previous studies have demonstrated that electrical stimulation of the nigrostriatal and mesolimbic DA neurons which contain autoreceptors results in an enhancement of tyrosine hydroxylation which is suppressed by DA agonists. Therefore, it was of interest to determine if mesocortical DA neurons responded to electrical stimulation in a similar fashion and, if so, how the stimulus induced activation of tyrosine hydroxylation would be influenced by DA agonists. If autoreceptors play a modulatory role in impulse regulated control of DA synthesis, systems which lack autoreceptors should be uninfluenced by autoreceptor agonists.

Electrical stimulation of the medial forebrain bundle (MFB) of rats anesthetized with chloral hydrate was carried out for 30 minutes with 0.4 mA biphasic pulses of 3.0 msec duration and 15 Hz. DOPA was isolated by ion exchange chromatography and subsequently measured by HPLC using electrochemical detection. A 30 minute DOPA accumulation after administration of a decarboxylase inhibitor (RO4-4602) was used as an index of *in vivo* tyrosine hydroxylation and dopamine synthesis. Unilateral electrical stimulation of the MFB caused significant increases in DA synthesis over sham stimulated controls in the striatum (+160%), olfactory tubercle (+120%), piriform cortex (+220%), prefrontal cortex (+55%), and cingulate cortex (+90%). Apomorphine administered at 0.1 mg/kg, i.v., reversed the stimulation-induced increase in DOPA accumulation by 80% or more in DA systems containing autoreceptors (i.e. the striatum, olfactory tubercle, and piriform cortex). No significant reversal by apomorphine was seen in the prefrontal and cingulate cortices.

This study demonstrates that increased impulse flow can enhance DA synthesis in mesocortical DA neurons. Furthermore, it supports the previous finding that functional autoreceptors are present in the allocortical projection innervating the olfactory tubercle and piriform cortex, but are absent in the neocortical projection innervating the prefrontal and cingulate cortices. Since the allocortex is phylogenetically older than the mesocortex and neocortex, the autoreceptors might represent a phylogenetically older mechanism for regulating DA synthesis. (Supported in part by USPHS Grant MH-14092 and the State of Connecticut).

291.20 DOPAMINE AGONISTS STIMULATE PROTEIN CARBOXYL METHYLATION IN STRIATAL SLICES, M.E. Wolf*, and R.H. Roth, (SPON: J.R. Cooper), Depts. Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

Protein carboxyl methylase (E.C. 2.1.1.24; PCM) catalyzes the formation of carboxyl methyl esters using S-adenosylmethionine (SAM) as a methyl donar. PCM activity is high in brain and has been implicated in presynaptic events including the modulation of neurotransmitter release. Studies utilizing striatal synaptosomes have suggested that PCM may be involved in autoreceptor mediated modulation of synaptic events. Apomorphine, as well as DA and the putative autoreceptor agonist 3-PPP, were found to increase methyl ester formation in striatal synaptosomes in a dose-dependent manner. The stimulatory effects of apomorphine could be blocked by DA antagonists and were not observed when synaptosomes were prepared from prefrontal cortex, a region devoid of DA autoreceptors (Billingsley and Roth, 1983).

We have extended our studies on PCM to striatal slices, a preparation in which it is easier to investigate the involvement of PCM in autoreceptor mediated modulation of DA synthesis and release. In this preparation we have demonstrated methyl ester formation in slices preloaded with ^{3}H-Methionine (^{3}H-MET). Slices were loaded with ^{3}H-MET rather than ^{3}H-SAM in order to avoid alteration in the pool size of SAM. Methyl ester formation was measured as the liberation of ^{3}H-Methanol from slice homogenates following alkaline hydrolysis with borate buffer. While uptake of ^{3}H-MET plateaued within 15 minutes, methyl ester formation increased in a linear manner from 15 to 90 minutes. ^{3}H-SAM was separated from ^{3}H-MET using SP-Sephadex chromatography. The appearance of ^{3}H-SAM preceded the formation of labeled methyl esters. Levels of ^{3}H-SAM increased linearly from 10 to 30 minutes before reaching a plateau.

Apomorphine (10^{-5}M) produced a significant increase in methyl ester formation when added to slices preloaded with ^{3}H-MET. This increase was blocked by the DA antagonist (+)-butaclamol. Previous studies using purified PCM have ruled out a direct activation of this enzyme by apomorphine. Additional experiments are underway to investigate the possible role of PCM in autoreceptor-mediated events. If apomorphine-stimulated PCM activity in slice preparations proves to be a reliable biochemical marker for the presence of DA autoreceptors, this technique may enable the study of DA autoreceptor distribution and function in primate brain. (Supported in part by USPHS grant MH-14092 and the State of Conn.)

291.PO EPINEPHRINE (E) NEURONS CONTROL PREOVULTORY LUTEINIZING HORMONE (LH) SECRETION IN THE RAT. S.P. Kalra* (spon: W.E. Brownell), Dept. OB-Gyn, University of Florida College of Medicine, Gainesville, FL 32610

E containing perikarya of the brain stem project into those specific sites in the preoptic area and hypothalamus including the median eminence which are extensively innervated by LH-releasing hormone producing neurons. These studies were aimed at examining the role of hypothalmic E in the preovulatory LH release. Rats displaying regular 4-day estrous cycles or ovariectomized rats were used for these studies. In the first experiment, proestrous rats received LY 78335 (2,3-dichloro-methyl-α-benzlamine, 50 mg/kg, ip) - a selective central inhibitor of phenylethanolamine N-methyl transferase which suppresses hypothalamic E without changing dopamine or norepinephrine (NE) levels - or saline (control) at 1200 and 1400 h. Blood samples were withdrawn from intrajugular cannulae at hourly intervals between 1400-1800 h for LH determination; occurrence of ovulation was checked the next day. While in control rats the preovulatory LH surge observed between 1400-1800 h induced normal ovulation (6/6, 8 eggs/rat), the afternoon LH surge and ovulation was completely blocked in LY 78335-treated rats. That a deficiency in hypothalamic E discharge may be responsible for blockade of LH surge was suggested by observations from the second experiment; intraventricular (Ivt) injections of E (5 µ/rat/3 µl) in similarly drug or saline-treated proestrous rats stimulated LH release. In the third experiment, the possibility that blockade of hypothalamic α-adrenergic receptors by LY 78335 may suppress LH surge, was examined. Ovariectomized rats bearing chronic intraventricular cannulae were primed with a sequential estrogen (10 µg/rat, 1000 h day 0) and progesterone (5 mg/rat, 1000 h day 2) treatment and received saline (control) or LY 78335 at 0800 and 1100 h on day 2. LH release in response to 2 Ivt injections, 1 h apart of E (5 µg/rat) or NE (5 µg/rat) beginning at 1200 h, was studied. Each Ivt E or NE injection elicited identical LH release in LY 78335-treated and control rats, thereby suggesting that suppression of LH release and ovulation after LY 78335 treatment could not be due to hypothalamic post-synaptic α-adrenergic receptor blockade. Thus, these studies implicate hypothalamic E innvervations to play a primary role in initiating the preovulatory LH discharge in rats. (Supported by NIH HD 08634).

292.1 MODULATION OF POPULATION RESPONSES TO PAIRED ORTHODROMIC PULSES, BY DOPAMINE, IN RAT HIPPOCAMPUS IN VITRO. Valentin K. Gribkoff, John H. Ashe, and Michael E. Lekawa*. Department of Psychology, University of California, Riverside, CA 92521.

Dopamine (DA) has recently been found to exist at significant levels in the rat hippocampus (Bischoff et al, Brain Res., 165:161, 1979; Ishikawa et al, Brain Res., 232:222, 1982), and to have direct effects on cell membrane characteristics (Bernardo and Prince, J. Neurosci., 2:415, 1982). In addition, DA has been shown to have a profound modulatory action on population and single neuronal responses to orthodromic stimuli (Gribkoff and Ashe, Soc. Neurosci. Absts., 8:483, 1982).

In the CA_1 region of the rat hippocampus, population responses to paired orthodromic volleys are characterized by: (a) at short inter-pulse-intervals (IPI's), the response to the second (test) stimulus is severely depressed, while (b) at longer IPI's significant facilitation of the test response is observed (Creager et al, J. Physiol., 299:409, 1980). In the present study, the population responses of CA_1 neurons, in vitro, to paired orthodromic stimuli were tested during and following DA application in order to determine the effects of DA on this form of synaptic plasticity.

DA (10^{-3}M), introduced into the recording chamber via push-pull cannulation, resulted in a significant increase in test response inhibition at IPI=20ms. Following removal of DA, there was a gradual return to near-control levels of inhibition.

DA (10^{-4}M) produced a small decrease in test response inhibition when tested at IPI=10-30ms, which was accompanied by a small decrease in peak levels of paired-pulse facilitation at IPI's greater than 40ms. Following removal of DA, a significant further decrease in the degree of inhibition was observed, being particularly pronounced at 120 min. following washout. The peak levels of paired-pulse facilitation were not significantly affected following removal of DA.

Inhibition of the test population response appears to be related to the activation of a feed-forward inhibitory pathway by the initial, i.e., conditioning, pulse (Haas and Rose, J. Physiol., 329:541, 1982). Both feed-forward and recurrent inhibition are presumably mediated by the release of gamma-amino butyric acid (GABA) (Alger and Nicoll, Nature, 281:315, 1979). The results of this study suggest that DA may exert a long-lasting influence on feed-forward GABA-nergic transmission in the rat hippocampus. (Supported by NIH grant BRSG-RR07010-17).

292.2 DOPAMINE DEPRESSES FREQUENCY OF EPILEPTIFORM BURSTING IN PYRAMIDAL NEURONS OF THE HIPPOCAMPUS IN VITRO. T. Suppes, A.R. Kriegstein* and D.A. Prince. Dept. of neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

The hippocampus receives presumptive dopaminergic input from brainstem regions. Dopamine (DA) application to hippocampal CA1 neurons in vitro causes hyperpolarization of resting potential, an associated increase in conductance, and an increase in both amplitude and duration of afterhyperpolarizations (AHPs) (Benardo and Prince, J. Neurosci. 2:415, 1982). We performed experiments to determine whether DA-induced effects would suppress epileptogenesis in the hippocampus. DA (40-160 μM) was focally applied to 450μ thick slices of guinea pig hippocampus prepared and maintained at 37°C using standard techniques. Epileptiform bursts were induced by adding penicillin (3.4 mM) to the perfusion medium.

Application of DA onto CA1 cells (n=15) produced a hyperpolarization averaging 4.5 mV beginning in 5-20 sec and lasting 2-3 min. DA also caused an increase in the amplitude and duration of slow AHPs. The frequency of spontaneous epileptiform events however was not affected by DA application to CA1. Since the pacemaker region for initiation of synchronous epileptiform discharges lies in the CA2-CA3 subfield (Wong, R.K.S. and Traub, R.D., J. Neurophysiol. 49:442-458, 1983; Schwartzkroin, P.A. and Prince, D.A., Ann. Neurol. 1:463-469, 1977), DA was focally applied to this region. CA3 neurons (n=6) responded to DA application with an initial 1-3 mV depolarization beginning within 15-30 sec and lasting 1-2 min. In two cases a small hyperpolarization lasting several min. subsequently developed. AHP duration and amplitude were increased 70% and 35% respectively (n=4). Along with these membrane changes the frequency of epileptiform bursting in CA3 cells slowed for 1-3 min.

These results suggested that a significant decrease in epileptiform burst frequency might occur in the follower CA1 region if greater numbers of pacemaker CA2 and CA3 cells were exposed to DA. We therefore added DA to the perfusion medium (10-80 μm) and recorded the frequency of penicillin-induced bursting in CA1. Spontaneous CA1 bursting was reversibly slowed, the interburst interval increased from a mean of 4 to a mean of 5 sec, and in some cases the interval at which bursts occurred became both slowed and much more variable. These changes in burst frequency could not be directly accounted for by increases in AHP duration.

The results of these experiments together with the demonstration of dopaminergic terminals in the CA2-CA3 region suggest that DA may play a role in decreasing the incidence or frequency of epileptogenic discharges in vivo.

Supported by NIH grant NS12151 from the NINCDS (DAP), a Klingenstein Foundation Fellowship (ARK) and a McCormick Postdoctoral Fellowship (TS).

292.3 IN VITRO ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF MIDBRAIN DOPAMINE NEURONS IN THE MOUSE. M.K. Sanghera, M.E. Trulson and D.C. German. Depts. of Psychiat. and Physiol., U. Texas Health Sci. Cntr., Dallas, TX 75235.

We have recently reported (Fed. Proc., 1983) that dopamine (DA)-containing neurons in the substantia nigra of the anesthetized mouse exhibit similar electrophysiological and pharmacological properties to those in the rat. That is, these cells have long duration action potentials (greater than 2 msec), fire from 2-8 impulses/sec, and the majority of cells exhibit a bursting firing pattern. This firing pattern is often characterized by spikes with a progressive decrease in amplitude within a burst. The purpose of the present experiment was to determine if these properties are endogenous to the DA cells or due to intra- and/or extranigral influences. Standard single cell recording and microiontophoresis techniques were employed. Recordings from nigral DA cells were made from midbrain slices containing (a) standard Yamamoto's solution and (b) modified Yamamoto's solution containing 0.5 mM Ca^{2+} and 30 mM Mg^{2+}. This medium has been shown to block all synaptic transmission. In vitro DA neurons exhibit similar electrophysiological and pharmacological properties to DA neurons in vivo. The cells had long duration action potentials (greater than 2 msec), fired from 4-10 impulses/sec, and had their firing rates decreased by the microiontophoretic application of DA and GABA. However, two main differences were noted. First, the mean (± SEM) firing rates of DA neurons in vitro was higher than in vivo (5.5 ± 0.4 vs. 3.9 ± 0.3 impulses/sec). Second, DA neurons in vitro often exhibited a pacemaker-like firing pattern, but when synaptic transmission was blocked, the cell routinely exhibited a pacemaker-like firing pattern. DA neurons in both in vitro preparations exhibited significantly less firing rate variation than in the in vivo preparation (e.g., coefficient of variation of the interspike-interval = 18 ± 3 msec for in vitro high Mg^{2+}/low Ca^{2+}; 22 ±5 msec for in vitro; 48 ± 8 msec for in vivo). These results suggest that the endogenous firing pattern of DA cells is pacemaker-like, similar to other monoaminergic neurons, and that the decremental bursting pattern seen in vivo is dependent upon both intra- and extranigral inputs.

Supported by grant MH-30546.

292.4 DOPAMINE RELEASED IN SUBSTANTIA NIGRA CAN MODULATE GABA-MEDIATED INHIBITION OF PARS RETICULATA NEURONS ELICITED BY STRIATAL STIMULATION. B.L. Waszczak and J.R. Walters. ETB, NINCDS, NIH, Bethesda, MD 20205.

Previous reports from this laboratory have described an ability of iontophoretically applied dopamine (DA) to attenuate the inhibitory responses of substantia nigra (SN) pars reticulata neurons to iontophoresed GABA. This finding raised the question of whether DA, released from SN dendrites, might act as a neuromodulator which diminishes responses of pars reticulata neurons to their striatal GABAergic innervation. These studies were conducted to determine whether iontophoresed DA, or endogenous DA released pharmacologically from SN dendrites, could attenuate the GABA-mediated inhibition of pars reticulata neurons which can be evoked by striatal stimulation.

Extracellular, single unit activity of SN pars reticulata neurons was recorded in male rats anesthetized with chloral hydrate. Stimuli (100-300 μA; 300 μsec duration) were delivered to the striatum at 1 Hz to the left striatum, ipsilateral to the recording site, through a 2 mm x 2 mm stimulating electrode array. Post-stimulus time histogram plots revealed that firing of pars reticulata neurons was inhibited with a short latency (8.2 ± 0.8 msec) for a discrete interval (26.0 ± 5.0 msec) following delivery of each stimulus. This inhibitory response to striatal stimulation was attributed to activation of striatonigral GABA pathways since it could be reduced by iontophoresis of the GABA antagonist bicuculline methochloride (5mM in 165 mM NaCl). After completion of three control post-stimulus time histograms, either DA (0.2 M, 10 nA ejection current) or d-amphetamine (AMPH; 0.2 M, 5-10 nA current) was applied iontophoretically for a period of 8-10 min. Histograms for three additional 1 min periods of striatal stimulation were determined during iontophoresis of each drug and compared with histograms from the control period.

Iontophoresed DA significantly ($p < .05$) reduced the ability of striatal stimulation to inhibit pars reticulata cell firing for 8 out of 18 cells tested (44%). This attenuation by applied DA was reflected by a 10-fold increase in the number of spikes occurring per msec bin during the inhibitory interval (0.05 ± 0.02 spikes/msec, control; 0.50 ± 0.11 spikes/msec during DA application). Similarly, AMPH, which has been reported to release DA from dendrites in the SN, was able to significantly attenuate the striatal-evoked inhibition of 8 out of 12 pars reticulata neurons (67%) when applied iontophoretically. The AMPH-induced attenuation was reflected by a 5-fold increase in the number of spikes occurring during the inhibitory interval (0.11 ± 0.05 spikes/msec, control; 0.57 ± 0.11 spikes/msec during AMPH application). For several cells it was also possible to significantly reduce the striatal-evoked inhibition of reticulata cell firing by i.v. administration of AMPH, 1.6 mg/kg.

These results provide evidence that the previously described modulatory interaction between DA and GABA can occur with physiological release of the two transmitters within the SN, supporting the idea that DA may directly influence SN pars reticulata output neurons.

292.5 THE EFFECT OF REPEATED HALOPERIDOL TREATMENT ON K^+ STIMULATED "RELEASE" IN THE RAT STRIATUM MEASURED BY IN VIVO ELECTROCHEMISTRY, J.O. Schenk* and B.S. Bunney. Depts. Psychiatry & Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510.

Previous studies have shown that the administration of haloperidol (HAL) has profound effects on the activity of dopaminergic (DA) cell bodies in the zona compacta (ZC) of the substantia nigra. Acute administration of HAL results in an increase in the firing rate and number of spontaneously active cells. Striatal concentrations of DA metabolites are elevated indicating increased turnover. Repeated administration of HAL results in an almost total loss of spontaneous DA cell activity. Intracellular studies have shown this loss of activity is accompanied by the development of depolarization block (DB). Under DB conditions the striatal levels of DA metabolites are close to control values, however, the effects of DB upon the dynamics of release in DA terminal areas have not been assessed.

The experiments were conducted in male Sprague-Dawley albino rats (300-490 gm). One group was administered HAL via their drinking water for 21 days prior to experimentation. The control group received no drug during this period. Each animal was given 75 mg/kg pargyline i.p. and anesthetized with chloral hydrate (400 mg/kg) before testing. A graphpoxy electrochemical electrode, calibrated in DA, was attached to a microsyringe containing 20% KCl and implanted in the striatum. The electrode-syringe separation was 500-900 um. Chronoamperometric measurements (1 sec on/60 sec off with E_{app} = 0.5 V vs. Ag/AgCl) were begun immediately. At baseline, depolarizations (DPOL) were initiated by injecting 1.0 ul of 20% KCl over a 30 sec period.

The magnitude of the signal observed after infusion of K^+ differed markedly between control and HAL treated animals. Responses in animals chronically treated with HAL were much lower (ca. 70%) compared to control animals. The signal in HAL animals was 48 (± 8 S.E.M.) uM DA (N=4 animals) while in controls the signal was 165 (± 14 S.E.M.) uM DA (N=4 animals).

Iontophoresis of GABA onto ZC DA neurons in DB has been shown to reverse DB and start them firing. Based on this data GABA was infused (1.0 ul 26 mM in 0.9% saline) into the ZC of HAL treated animals prior to K^+ DPOL of the striatum to test the relationship between DB and the observed change in release. The K^+ elicited signal obtained in the striatum after GABA infusion was 187 (± 27 S.E.M. uM DA (N=3 animals). These K^+ induced signals are similar to controls. Combined, these data suggest that the DA cell state of DB induced by repeated HAL treatment produced a marked change in the ability of K^+-DPOL to induce release. Striatal DA cell terminals may then be in a state of tonic DPOL secondary to somatic DB. (Supported by PHS Grants MH-14276, MH-28849 and the State of CT.)

292.6 SINGLE SPIKING AND BURST FIRING IN NIGRAL DOPAMINE NEURONS. A.A. Grace & B.S. Bunney, Depts. Psychiatry & Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06510.

Dopamine neurons fire action potentials in one of two patterns: 1) irregular single spikes, or 2) burst firing. Intracellular and extracellular recordings of identified nigral dopamine (DA) neurons were performed in vivo in the rat to elucidate the ionic mechanisms regulating each firing pattern.

In the single spike firing pattern, DA cells fire spikes in a slow, irregular pattern with interspike intervals typically 200-300 mSec. Intracellular recording reveals that this pattern is mediated by an alternating voltage dependent slow pacemaker-like depolarization (S_v, 78 ± 40 mSec, 13 ± 3 mV, Mean ± SD) and a post-spike calcium activated potassium current ($I_{K(Ca)}$). The S_v rate of rise increases with depolarization, and can be truncated by a short hyperpolarizing current pulse. This current is specifically inhibited by i.v. apomorphine. The $I_{K(Ca)}$ occurs only after somatodendritic (SD) spikes and its amplitude is proportional to the number of spikes elicited by depolarization. $I_{K(Ca)}$ can be eliminated with EGTA or long-term (30 min) TEA injection. EGTA also blocks the accomodation in firing rate occurring during depolarizing pulses (150 mSec duration). Furthermore, EGTA injection increases spike amplitude by almost 20 mV (up to 90 mV amplitude), possibly by removing a high resting calcium inactivation.

DA neurons are also observed to fire in a bursting pattern of 3-10 spikes with an interspike interval of 73 ± 13 mSec. As the burst progresses, each successive spike occurs with a longer interspike interval, as well as increasing duration and decreasing amplitude. 75% of the 75 DA neurons sampled were burst firing (at least 2 three spike bursts per 500 spikes), with an average 15% of the spikes occurring in bursts. Although the degree of burst firing is not correlated with baseline firing rate (r=.37), bursting increases linearly with increasing firing rate produced by glutamate iontophoresis or long term (>5 min) depolarizing current injection. Evidence that burst firing is dependent on calcium entry during spiking includes: 1) Intracellular calcium injection causes burst firing independent of membrane depolarization, and 2) EGTA blocks depolarization-elicited burst firing. The calcium entry is thought to activate burst firing by decreasing a voltage activated potassium current ($I_{K(v)}$) because: 1) depolarization initially decreases input resistance, but this resistance returns to baseline with continued depolarization, 2) TEA injection leads to burst firing, and 3) barium iontophoresis increases burst firing. (USPHS MH28849,MH25642 & State of Connecticut).

292.7 STRIATAL INFUSION OF DOPAMINERGIC AGENTS AFFECTS ORTHODROMIC AND ANTIDROMIC EXCITABILITY OF NIGRAL DOPAMINERGIC NEURONS J.M. Tepper*, S.J. Young*, and P.M. Groves. Univ. of Calif. San Diego, Dept. Psychiatry M-003, La Jolla, CA 92093.

The existence of dopamine receptors located on or near the terminals of nigrostriatal dopamine neurons has been inferred from studies showing that stimulus-evoked release of dopamine as well as its biosynthesis can be inhibited by dopamine agonists and enhanced by dopamine antagonists. We have previously suggested that pharmacological stimulation of the terminal autoreceptor decreases dopamine terminal excitability while blockade increases excitability. The results reported here were obtained by measuring the current necessary to elicit antidromic responses in nigral dopaminergic neurons in urethane anesthetized, immobililized and artificially ventilated rats from neostriatal stimulation.

The magnitude of the decrease in dopaminergic terminal excitability following striatal amphetamine infusions (.31 ul,10 uM) was inversely correlated with the pre-drug firing rate of the cell (r=-.61,df=13,p<.05). The magnitude of the increase in terminal excitability following neostriatal infusions of haloperidol (.31 ul,0.1-1.0 uM) was positively correlated with the pre-drug firing rates (r=.45,df=15,p<.05). Terminal excitability was also decreased by stimulation of the medial forebrain bundle (MFB) with 750 ms trains at 2-10 Hz. These decreases were inversely correlated with pre-stimulation firing rates (r=-.66,df=13,p<.05). These results imply that under physiological conditions, the rate of impulses reaching the terminal regions influences terminal excitability, and by inference, the amount of dopamine released per impulse.

Striatal injection of kainic acid (.625 ug in .5 ul), did not impair the ability of apomorphine or MFB conditioning stimuli to reversibly decrease terminal excitability despite a near total destruction of intrinsic striatal neurons around the injection and stimulation-infusion site.

Striatal infusions of amphetamine led to decreases (t=4.12,df=11,p<.05) while haloperidol produced increases (t=3.46,df=11,p<.05) in the proportion of antidromic spikes that invaded the soma-dendritic regions of the cell. These effects were independent of the modest increases and decreases in nigral firing rate following neostriatal infusions of amphetamine and haloperidol, respectively. This effect may be attributable to complex orthodromic synaptic effects which could lead to independent modulation of initial segment and soma-dendritic excitability. (This research was supported, in part, by grants DA-K01-02854 and DA-K02-00079 to P.M.G.)

292.8 ACTIONS OF THE 3-PPP ENANTIOMERS ON NIGRAL DOPAMINE (DA) NEURONS. I. SYSTEMIC STUDIES. D. Clark*, G. Engberg*, T.H. Svensson and A. Carlsson* (SPON: S. Grillner). Dept. of Pharmacology, University of Göteborg, Göteborg, Sweden.

Previous biochemical and behavioural experiments indicate that the enantiomers of the DA analogue 3-(3-hydroxyphenyl)-N-n-propylpiperidine, 3-PPP, are active on different DA receptors in brain. (+)-3-PPP appears to act as a classical DA agonist, i.e. activating both post- and autoreceptor sites. In contrast, (-)-3-PPP stimulates DA autoreceptors but also blocks postsynaptic DA receptors. Here we report the effects of systemic administration of the two enantiomers on DA- and non-DA neurons in substantia nigra of the rat as revealed by single cell recording techniques in chloral hydrate anaesthetised rats.

The inhibitory actions of both compounds on DA neuronal firing rate were not blocked by depletion of endogenous DA, arguing against an indirect mode of action of the drugs. Furthermore, non-DA neurons in substantia nigra, i.e. in zona reticulata, were not significantly affected by doses of the drugs which consistently inhibited DA neurons in z. compacta showing specificity of action. All DA neurons encountered, as identified by electrophysiological characteristics and conventional histology, were dose-dependently inhibited by intravenously (i.v.) administered (+)-3-PPP, an effect which was readily and totally antagonized by i.v. injected haloperidol. At least two types of DA neuronal responses to i.v. administered (-)-3-PPP were observed: Cells with a low baseline firing rate (<3 spikes/sec) were inhibited exactly as after the dextroenantiomer. Cells with a high baseline firing rate (>4 spikes/sec) were only inhibited to about 50 per cent by (-)-3-PPP, regardless of dose. Both actions were reversed by i.v. haloperidol.

Based on these and previous experiments, it is hypothesized that the failure of (-)-3-PPP to completely inhibit the fast DA neurons may in part be related to its postsynaptic DA receptor blocking action. Alternatively, a local regulatory circuitry within the nigra may be blocked (Svensson et al., this meeting). The two nigral DA cell populations observed here may differ with respect to their regulatory mechanisms and this possibility is currently being investigated.

292.9 ACTIONS OF THE 3-PPP ENANTIOMERS ON NIGRAL DOPAMINE (DA) NEURONS. II. MICROIONTOPHORETIC STUDIES. T.H. Svensson, G. Engberg*, D. Clark*, A. Carlsson*. Dept. of Pharmacology, University of Göteborg, Göteborg, Sweden.

Previous biochemical and behavioural experiments have indicated that the dextro-enantiomer of 3-(3-hydroxyphenyl)-N-n-propyl piperidine, (+)-3-PPP, acts as a classical DA receptor agonist on both autoreceptors and postsynaptic receptors. Furthermore, the data provide evidence that (-)-3-PPP acts as an agonist on DA autoreceptors but as an antagonist on postsynaptic receptors. Here we report the effects of microiontophoretically applied (+)- and (-)-3-PPP on A_9 DA neurons.

In agreement with the effect of intravenously administered (+)-3-PPP (Clark et al., this meeting), its microiontophoretic applications as well as that of DA produced inhibition of all A_9 cells tested. This action was antagonized by concomitant application of a DA receptor antagonist (trifluoroperazine, TFP), analogously to our systemic results. Iontophoresis of (-)-3-PPP produced an inhibition or had no effect on firing of slow cells (< 3 spikes/sec). On fast DA cells (> 4 spikes/sec) in the substantia nigra, however, (-)-3-PPP either slightly increased firing rate or had no effect, contrasting with the consistent inhibition produced by DA. Furthermore, the inhibitory action on the fast cells of DA or (+)-3-PPP was antagonized by simultaneously applied (-)-3-PPP.

These data indicate that (+)-3-PPP acts as a DA-receptor agonist on autoreceptors within the substantia nigra on or in close vicinity of the cell bodies. Moreover, (-)-3-PPP appears to exert the same action on a population of slow A_9 neurons. However, the antagonist effect of (-)-3-PPP on the fast cells could indicate a second type of DA receptor on or near some DA neurons in substantia nigra. The autoreceptor activity of (+)-3-PPP and, on slow A_9 neurons, of (-)-3-PPP can explain the inhibitory action on the cells of systemic administration of the enantiomers. The failure of systemically administered (-)-3-PPP to completely inhibit the fast cells may be related to its antagonistic action on DA receptors of classical postsynaptic type or, as suggested here, by the same effect on DA receptors located near or on the A_9 cell bodies, possibly part of a feedback loop intrinsic to substantia nigra.

292.10 ELECTRICAL STIMULATION OF THE LATERAL HABENULA INHIBITS SINGLE DOPAMINE-CONTAINING NEURONS IN THE SUBSTANTIA NIGRA AND VENTRAL TEGMENTAL AREA. G. R. Christoph*, R. J. Leonzio*, and K. S. Wilcox* (SPON: W. F. Herblin). Central Res. & Dev. Dept., E. I. du Pont de Nemours & Co., Wilmington, DE 19898.

A subgroup of neurons in the lateral habenula have efferent projections that terminate in the substantia nigra pars compacta (SNC) and ventral tegmental area (VTA) where cell bodies of dopamine (DA)-containing neurons are located. In order to study the influence of the habenula on dopaminergic activity, single-unit electrophysiological techniques were used to record unit discharge of presumed DA-containing neurons in the SNC and VTA during electrical stimulation of the lateral habenula or adjacent structures. In chloral hydrate anesthetized rats, glass microelectrodes were stereotaxically positioned within the SNC and VTA. DA-containing neurons were identified on-line by their characteristic spike duration (>2 msec), discharge rate (2-8 spikes/sec), and irregular firing pattern. Typically, 5 neurons were tested per rat. Analysis of peri-stimulus time histograms showed that 85% of SNC cells (N=50) and 95% of VTA neurons (N=44) were inhibited after single pulse stimulation (0.25 mA, 0.1 msec) of the lateral habenula. The mean time between stimulation and onset of inhibition was 11 msec (range = 2-22 msec) and mean duration of maximal suppression was 70 msec (range = 20-250 msec). Approximately 2/3 of SNC and VTA neurons showed excitation after inhibition. Stimulation of structures adjacent to the lateral habenula (hippocampus, lateral thalamus, medial habenula) had no effect or greatly attenuated effects on DA-containing neurons. Electrolytic lesions of the fasciculus retroflexus, the fiber pathway which contains habenular efferents, blocked the habenular stimulation effects on all 20 DA-containing neurons tested. Electrolytic lesion of the stria medularis, which contains habenular afferents, did not alter the inhibitory effect of habenular stimulation (N=20). Cytotoxic kainic acid injections in the lateral habenula (250 nl, 1 ngm/nl) one week before recording sessions blocked the inhibitory consequences of habenular stimulation (N=25). Fink silver stains were used to histologically visualize kainate-induced cellular damage and subsequent degeneration in the lateral habenula and fasciculus retroflexus. Degeneration in the stria medularis was not detected. These experiments indicate that activation of neuronal cell bodies in the lateral habenula causes orthodromic inhibition on DA-containing neurons in SNC and VTA via the fasciculus retroflexus.

292.11 DOPAMINERGIC EFFECTS ON SUBSTANTIA NIGRA ZONA RETICULATA NEURONS. R.T. Matthews and D.C. German. Depts. of Physiol. and Psychiat., U. Texas Health Sci. Cntr., Dallas, TX 75235.

The substantia nigra zona reticulata (ZR) contains cell bodies, many of which contain GABA, and dopamine (DA)-containing dendrites. These dendrites release DA into the ZR and influence cell firing. Previous studies have shown that microiontophoresed DA (a) excites ZR neurons (Ruffieux & Schultz, Nature, 285:240-241, 1980), and (b) attenuates GABA inhibition of ZR neuronal impulse flow (Waszczak & Walters, Science, 220:218-221, 1983). The substantia nigra is known to contain DA autoreceptors (located on DA neurons), D1 receptors (adenylate cyclase-linked receptors) and D2 receptors (non-adenylate cyclase linked receptors).

The purpose of the present series of experiments was to compare the response properties of DA receptors on the DA neuron (the DA autoreceptor) with the DA receptors on the ZR neurons. Standard single cell recording and microiontophoresis techniques were employed in chloral hydrate anesthetized rats. ZR cells were identified on the basis of their firing rates (10-40 impulses/sec), < 2 msec biphasic action potentials, and location below DA neurons and within the ZR.

We found that: (1) ZR cells were increased in rate by iontophoresed DA (20-40 nA); (2) these same cells were not affected by systemic apomorphine (APO; 10 µg/kg i.v.), although this dose routinely decreases DA neuronal impulse flow; (3) a low dose of d-amphetamine (d-AMP; 1 mg/kg i.v.) also had no affect on impulse flow but 10 mg/kg tended to increase ZR cell firing and this affect was reversed by haloperidol (0.5 mg/kg i.v.); (4) both microiontophoresed DA and APO increased ZR cell firing in a comparable fashion to their ability to decrease DA neuronal cell firing; and (5) cis-flupenthixol blocks the DA excitation of ZR cells more effectively than haloperidol. These data will be discussed in terms of possible ZR DA receptor subtypes.

Supported by grant MH-30546.

292.12 SEXUAL DIFFERENCES IN THE RESPONSE OF TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS TO ACUTE RESTRAINT STRESS. G.D. Riegle*, K.E. Moore and K.T. Demarest (SPON: W. D. Atchison). Depts. of Physiology and Pharmacology/Toxicology, Michigan State Univ., East Lansing, MI 48824.

Previous studies have demonstrated a fundamental sexual difference in the activity of tuberoinfundibular dopaminergic (TIDA) neurons; female rats have a higher rate of DA synthesis and turnover in the median eminence (ME) than do males (Neuroendocrinology 32: 108, 1981). This difference may be due, in part, to an increased sensitivity of TIDA neurons in the female to the actions of prolactin (Neuroendocrinology 33: 230, 1981). In the female rat, physiologic increases in prolactin secretion (estrous cycle, pregnancy, lactation) are associated with decreases in TIDA neuronal activity (Neuroendocrinology 32: 24, 1981; 34: 229, 1982; 36: 130, 1983); these changes may represent a sexual difference in the neuronal circuitry regulating these neurons. In both sexes, stress induces a rapid increase in prolactin secretion.

The present studies were undertaken to examine the role of TIDA neurons in the stress-induced increase in prolactin secretion in male and female rats and to evaluate the use of this model to study afferent neuronal influences on TIDA neurons. Intact male rats, female rats on the second day of diestrus, and two week ovariectomized rats were used. TIDA neuronal activity was estimated by measuring the in vivo rate of DA synthesis in the ME (the rate of DOPA accumulation 30 min after NSD 1015, 100 mg/kg, i.p.). The stress consisted of 30 minutes of immobilization. Serum prolactin concentrations increased in both the male and female rats after 30 min of stress but the magnitude of the response was greater in the female. In the male, prolactin concentrations returned to control levels 1 h after the onset of stress, while in the female the stress-induced increase was maintained. The stress did not alter the rate of DOPA accumulation in the ME of male rats, but caused a dramatic decrease in both groups of female rats. These stress-induced changes in DOPA accumulation in the ME of the female were specific in that no changes were observed in other DA terminal regions (i.e., striatum and posterior pituitary).

These results suggest that there are sexual differences in the response of TIDA neurons to acute restraint stress. This difference may be a consequence of differences in neuronal circuits, endogenous hormonal milieu or the basal rate of TIDA neuronal activity in male and female rats. (Supported in part by USPHS grants AG02644 NS09174.)

292.13 THE RESPONSIVENESS OF TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS TO PROLACTIN IS DETERMINED BY PRECEDING CIRCULATING CONCENTRATIONS OF THIS HORMONE. K.T. Demarest, G.D. Riegle* and K.E. Moore. Depts. of Pharmacol./Toxicol. and Physiol., Mich. State Univ., E.Lansing, MI 48824.

There are two components of the prolactin-induced activation of tuberoinfundibular dopaminergic (TIDA) neurons: 1) a rapid 'tonic' component which responds to acute changes in circulating prolactin and maintains the "basal" activity of these neurons and 2) a delayed "induction" component which responds to prolonged elevations of prolactin and determines the responsiveness of the "tonic" component (Demarest and Moore, Fed. Proc. 42: 1155, 1983). The following studies were undertaken to determine if long-term decreases in circulating prolactin reduce the responsiveness of TIDA neurons to this hormone. The activation of TIDA neurons by prolactin was determined at various times after hypophysectomy or after acute or chronic treatment with bromocriptine (2 mg/kg/day, s.c.) by measuring the rate of DA synthesis (DOPA accumulation 30 min after NSD 1015, 100 mg/kg, i.p.) in the median eminence (ME) of ovariectomized rats. Twenty-four hours after hypophysectomy or the first injection of bromocriptine the rate of DA synthesis in the ME was decreased; this decrease was maintained for at least 12 days suggesting that TIDA neuronal activity is normally maintained by endogenous prolactin. In addition, chronic reduction of prolactin reduces the responsiveness of TIDA neurons to the acute stimulatory action of this hormone. That is, i.c.v. prolactin (10 µg, 12 hr prior to sacrifice) caused a similar increase in the rate of DA synthesis in the ME of control, 24 hr hypophysectomized and 24 hr bromocriptine-treated rats. On the other hand, after longer periods (6-12 days) of bromocriptine treatment or after hypophysectomy the responsiveness of TIDA neurons to prolactin was markedly reduced. For example, dose-response studies in 12 day hypophysectomized rats revealed a decrease in the sensitivity and magnitude of response to i.c.v. prolactin. This decreased responsiveness of TIDA neurons to the actions of prolactin following long-term bromocriptine treatment and hypophysectomy is most likely due to the prolonged decrease in circulating prolactin, since the daily concurrent administration of bromocriptine and i.c.v. prolactin for 12 days maintained the ability of TIDA neurons to respond to prolactin. These results suggest that the response of TIDA neurons to prolactin is determined by the preceding circulating levels of this hormone with prolonged increases enhancing and prolonged decreases reducing the acute response of TIDA neurons to prolactin. (Supported by USPHS grants NS09174 and AG02644.)

292.14 LONG-TERM TREATMENT WITH ESTRADIOL INDUCES A REVERSIBLE DECREASE IN THE RESPONSIVENESS OF TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS TO PROLACTIN. K.E. Moore, G.D. Riegle* and K.T. Demarest. Depts. of Pharmacol./Toxicol. and Physiol., Mich. State Univ., E. Lansing, MI 48824.

Short-term treatment with estradiol increases the rate of turnover and synthesis of dopamine (DA) in terminals of tuberoinfundibular (TI) neurons in the median eminence (ME) by virtue of the ability of this hormone to increase circulating concentrations of prolactin (Eikenburg et al., 1977; Demarest and Moore, 1980). The present studies were undertaken to examine the long-term effects of estradiol on serum prolactin concentrations and TIDA neuronal activity (estimated by the rate of DOPA accumulation in the ME 30 min after NSD 1015, 100 mg/kg, i.p.). Female rats, ovariectomized for 2 weeks, were implanted s.c. with silastic capsules containing estradiol benzoate, which maintained serum concentrations of this hormone from 60 to 80 pg/ml. The animals were sacrificed 6 and 18 days after capsule implantation. Serum prolactin concentrations were markedly increased at both times whereas the rate of DOPA accumulation was increased at 6 days but not at 18 days. The concentration of DA in the ME was reduced at 6 days and further reduced at 18 days. The low rate of DOPA accumulation in the ME despite the high circulating concentrations of prolactin suggest that long-term estradiol treatment reduces the ability of TIDA to respond to prolactin. This was confirmed by the finding that direct i.c.v. injections of prolactin (10 µg, 12 hr prior to sacrifice) increased the rate of DOPA accumulation in the ME of sham-implanted rats but not in 18 day estradiol-treated rats.

To determine if the effects of estradiol were reversible, ovariectomized rats were implanted with empty or estradiol-containing capsules for 18 days. The capsules were then removed and the animals sacrificed at various times up to 18 days later. The elevated serum concentration of prolactin and the reduced DA concentration in the ME of the chronic estradiol-treated rats returned to control values within 18 days after removing the capsules. On the other hand, the rate of DOPA accumulation in the median eminence increased progressively after removing the capsules reaching a maximum by 18 days. These results suggest that there may be a rebound increase in the sensitivity of TIDA neurons to prolactin in rats that had the estradiol capsules removed. This was substantiated by the fact that i.c.v. prolactin caused a greater increase in the rate of DOPA accumulation in the post-estradiol group of rats than in the sham controls.

The results of these studies suggest that long-term estradiol treatment reduces the activity of TIDA neurons, in part by attenuating their responsiveness to prolactin, and that termination of estradiol treatment results in a rebound increase in the sensitivity of TIDA neurons to prolactin. (Supported by NIH grants NS09174 and AG02644, and a PMAF Starter Grant to KTD.)

292.15 DOPAMINE RECEPTOR-MEDIATED POTENTIAL CHANGES OF FROG AFFERENT FIBERS. G.P. Ryan*, J.C. Hackman, C.J. Wohlberg & R.A. Davidoff. (SPON: P. Scheinberg). Neurophysiology Laboratory, VA Medical Center & Depts. of Neurology & Pharmacology, Univ. of Miami School of Medicine, Miami, FL 33101.

To elucidate the role of dopamine (DA) in spinal cord function, the effects of the substance on afferent fiber membrane potential was measured. The isolated hemisected frog spinal cord continuously superfused with Ringer's solution at 15°C was used for this purpose. The membrane potential of primary afferent fibers contained in the ninth dorsal root (DR) was recorded by use of the sucrose gap method.

The most prominent effect of DA was a slow reversible hyperpolarization, an action consistently observed at concentrations > 0.01 µM. The amplitude and duration of the hyperpolarization was dependent upon the concentration and duration of DA application. In 10 cords exposed to 10 µM DA for 30 sec the hyperpolarization averaged 0.93 mV. The DA-elicited hyperpolarization was reproducible upon repeated applications at 10 minute intervals. When DA was applied at higher concentrations or for longer durations, a biphasic response was produced with an initial dominant hyperpolarization followed by a smaller depolarization.

The application of SKF 38393A, a selective D-1 agonist and LY-14186, a selective D-2 agonist produced DR hyperpolarizations similar to those of DA, but only when applied at higher concentrations (1 mM). SKF 38393A was slightly more potent than LY 141865.

The nonselective DA antagonists fluphenazine (10 µM) and haloperidol (10 µM) reversibly blocked the effects of DA. The selective D-2 antagonists sulpiride and metoclopramide, acted similarly and in concentrations (10 µM) which had no effect on the hyperpolarization produced by 10 µM norepinephrine (NE).

To test the hypothesis that the DA induced hyperpolarization might involve adrenergic receptor activation we applied the adrenergic antagonists, yohimbine (1 µM)-an α_2-antagonist, corynanthine (1 µM)-an α_1-antagonist, and propanolol (10 µM)- a β-antagonist. These were without effect on the hyperpolarizations produced by 10 µM DA, but yohimbine blocked NE hyperpolarizations.

Superfusion of the cord with Ringer's containing tetrodotoxin (1.25 µM) or Mn^{++} (1.5. mM), suppressed reflex activity and significantly reduced the DA hyperpolarization. These results indicate that the DA effect is the result of both a direct action on primary afferent terminals and an indirect effect on interneurons.

These data show that DA has the ability to activate specific receptors on both interneurons and on afferent terminals. Such activation changes the membrane potential of afferent terminals and may play a role in sensory transmission in the spinal cord.

Supported by VAMC Funds # 1769 and USPHS grant # NS 17577 and HL 07188 and a grant from the National Parkinson Foundation.

292.16 FUNCTIONAL MAPPING OF EFFECTS OF DOPAMINE RECEPTOR BLOCKADE UPON LIMBIC FOREBRAIN AND MIDBRAIN OF THE RAT. P. Ramm*, R.J. Beninger and B.J. Frost.

We have examined the effects of dopamine (DA) receptor blockers (pimozide, alpha-flupenthixol, metaclopramide) upon local cerebral glucose utilization (LCGU) in origin and termination areas of primary pathways connecting the limbic forebrain and the midbrain. A ventral system arising in anterior olfactory areas travels caudally to terminate in the ventral tegmental, lateral preoptic and lateral hypothalamic regions. A dorsal diencephalic conduction system originates from anterior portions of the medial forebrain bundle and terminates in the habenula. Efferent fibers from the lateral habenula (LHb) terminate in a number of sites including the ventral tegmentum, substantia nigra, central gray, mesencephalic reticular formation and dorsal and ventral raphe nuclei.

Rats received an IP injection of one of the DA receptor blockers or of saline. Under halothane anesthesia, the tail artery and vein were cannulated. Three hours later, the alert immobilized animals were injected with 50 uCi/kg of ^{14}C-2-deoxyglucose arterial blood samples were taken during the subsequent 45 min., and the brains were then cut at 20u and autoradiographs prepared.

The effects of DA receptor blockade were similar with all drug treatments. The treated animals (N=9) were, therefore, combined for comparison with controls (N=3).

The most obvious effect of DA receptor blockade was greatly increased LCGU in the LHb. This finding is consistent with a previous report of increased glucose utilization in LHb following interference with DA function (Wooten & Collins, J. Neurosci. 1, 285, 1981). Limbic and striatal regions receiving DA afferents are known to converge on the LHb. The present findings suggest that a major consequence of treatments with DA receptor blockers is to alter the influence of these important emotion and motor circuits on descending pathways, via the LHb.

292.17 MICROINJECTION OF 6-HYDROXYDOPAMINE INTO THE REGION OF THE NUCLEUS TRACTUS SOLITARIUS: EFFECTS ON SLEEP-WAKING*
Bronzino, J.D., C. Siok* and P.J. Morgane, Trinity College, Hartford CT 06106

The region of the nucleus tractus solitarius (NTS) has been reported to be rich in catecholomine terminals and send significant projections to the locus coeruleus. The locus coeruleus, in turn, is a major contributor to the reticulo-septo-hippocampal and cerulo-cortical systems and has been implicated in the maintenance of the waking and REM sleep portions of the sleep cycle. The present study was initiated to determine whether injection of a catecholomine specific lesioning agent, 6-Hydroxydopamine (6-OHDA), into the region of the NTS would alter the normal sleep-waking patterns of the rat. Ten days after surgery was performed on male, Sprague Dawley retired breeder rats, EEG recordings were obtained over a 4 hour period on three different days to establish the sleep-waking profile for each animal. At the end of the baseline recording sessions, all animals were injected with 8 ug of 6-OHDA dissolved in buffered Ringer's lactate containing 0.1% ascorbic acid in a total volume of 0.15 ul over a 30 second period. All injections were made through a 33 gauge cannula system designed to extend 1/2 mm beyond the tip of the cannula guide. Sleep-waking recording sessions were conducted at 5 and 10 days post injections, and comparisons made directly to each animal's baseline recordings.

Our preliminary results indicate that the most significant changes brought about by injection of 6-OHDA in the region of the NTS were: (1) a significant increase in the percent of time spent in slow-wave sleep, (2) a significant decrease in the amount of time spent in the waking state (active and quiet, combined), (3) an increase in the percent of time spent in REM sleep, and (4) an increase in the total number of REM episodes. The control animals, i.e., those with cannula placements outside the region of the NTS on the other hand, showed no significant changes in sleep ,behavior. These preliminary results suggest that the catecholomine terminals in the NTS do play a role in sleep-waking activity.

*Supported by NSF Grant ECS-8118440

292.18 AN ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL EXAMINATION OF A10 DOPAMINE AUTORECEPTORS. P.D. Shepard* and D.C. German. (SPON: L. Hersh). Depts. of Physiol. and Psychiat., U. Texas Health Sci. Cntr., Dallas, TX 75235.

The ventral tegmental area (nucleus A10) dopamine (DA)-containing neurons have been the focus of anatomical, electrophysiological and pharmacological examination. Specific groups of these A10 neurons project to limbic (nucleus accumbens - NA and olfactory tubercle) and cortical (prefrontal - PF, cingulate - CG, and entorhinal) regions. These neurons have been reported to fire slightly faster than the nigrostriatal (A9) DA neurons (greater than 4 impulses/sec on the average) and exhibit long duration action potentials with decremental burst firing patterns. The A9 neurons possess cell body DA autoreceptors, whose activation by iotophoretic DA or systemic apomorphine (APO) decreases impulse flow. However, the PF and CG projecting A10 cells have been reported not to possess cell body autoreceptors (Bannon et al, Neurosci. Abstr. 8:480, 1982). These neurons were not decreased in firing rate by locally applied DA or systemically administered APO. Other investigators have found evidence to suggest that d-amphetamine (d-AMP) decreases A10 DA neuronal impulse flow by dendritic release of DA onto DA autoreceptors (Dalsass et al, Neurosci. Abstr., 5:553, 1979 for mesolimbic A10 neurons; Wang, Brain Res. Rev., 3:153-165, 1981 for mesolimbic and mesocortical A10 neurons). If mesocortical DA neurons lack cell body autoreceptors, how can d-AMP decrease impulse flow in these neurons?

The present experiment sought to examine the electrophysiological and pharmacological properties of A10 DA neuron autoreceptors. Single cell recording and microiontophoretic techniques were used in chloral hydrate anesthetized rats. Our preliminary results indicate that A10 cells (a) antidromically activated from the NA fire at similar rates to A9 neurons, and these A10 cells are decreased in rate by iontophoretically applied DA and systemically administered APO (5-10 μg/kg i.v.) and d-AMP (0.5-1.0 mg/kg i.v.); and (b) are less sensitive to presynaptic doses of systemically administered APO (5 μg/kg i.v.) and microiontophoresed DA (5-20 nA) than are A9 cells. Experiments are in progress to examine the responses of PF and CG A10 neurons to APO and d-AMP.

Research supported by grant MH-30546.

292.19 CHRONIC d-AMPHETAMINE TREATMENT REDUCES THE AUTOREGULATORY ABILITY OF A10 DOPAMINE NEURONS. F.J. White and R.Y. Wang. Dept. of Pharmacology, St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Chronic abuse of the dopamine (DA) agonist d-amphetamine (AMP) often leads to clinical syndromes resembling paranoid schizophrenia (AMP psychosis). This fact has become an essential cornerstone of the DA hypothesis of schizophrenia which postulates hyperactive mesolimbic and mesocortical (A10) DA systems as a pathophysiological factor. In animals; chronic AMP (CAMP) leads to both enhanced and diminished behavioral responses to acute AMP challenge; the enhanced responses (sensitization) most closely parallel the clinical situation since AMP-psychosis usually is manifest only after CAMP abuse. The neuronal mechanisms responsible for sensitization remain to be elucidated. In the A10 DA systems, AMP-stimulated DA release is coupled to a compensatory decrease in the activity of A10 DA neurons which is mediated by self-inhibitory autoregulatory mechanisms (Wang, Brain Res. Rev. 3:153, 1981). In the present experiments, we tested the hypothesis that CAMP induced sensitization of A10 DA systems is mediated by a decreased autoregulatory ability of A10 DA neurons.

Groups of male rats received either of two dose regimens of d-AMP sulphate (ip), 1X5 mg/kg/day or 2X5 mg/kg/day, for seven days. Control rats received daily injections of saline. Single unit recordings of identified A10 DA neurons were obtained 24-32 hrs after the last injection. The sensitivity of DA autoreceptors was assayed using 3 challenge techniques: by increasing iv doses of d-AMP; by low "autoreceptor" doses (iv) of apomorphine (APO); and by direct iontophoretic (IONTO) application of DA (0.1 M). Both CAMP regimens significantly increased the doses of AMP and APO required to suppress the firing of A10 DA cells. In fact, at every dose of AMP and APO, the responsiveness of A10 DA cells in CAMP rats was significantly reduced as compared to control rats. There were no significant differences between the two CAMP groups. The ID_{50} for AMP suppression of A10 activity was 0.48 mg/kg in control rats as compared to 1.99 and 2.12 mg/kg in the 1X and 2X CAMP groups, respectively. The ID_{50}'s for APO suppression were 0.007 (control), 0.021 (1X) and 0.03 mg/kg (2X). Preliminary results from IONTO-DA tests indicated that the mean suppression of A10 activity induced by 10 nA DA was 16% in 2X rats (n=10 cells) as compared to 48% in control rats (n=24 cells). These results demonstrate that CAMP caused a 3-4 fold shift to the right in dose-response curves for DA agonist inhibition of A10 DA activity. This subsensitivity of A10 DA autoreceptors (located on or near the cell body) may be responsible for CAMP-induced behavioral sensitization and AMP psychosis. (Supported by USPHS Grants MH34424 and MH00378 and the Scottish Rite Schizophrenia Research Program NMJ, USA.)

292.20 DIFFERENT RESPONSES OF SN-DA NEURONAL ACTIVITY TO PSYCHOTROPIC DRUGS IN UNANESTHETIZED AND ANESTHETIZED RATS
G.P. Mereu* (SPON: W. Fratta). Institute of Biology, University of Cagliari, Via Porcell, 2 - 09100 Cagliari, Italy.

Since 1965 (1), the anesthetic-induced differences in CNS neuronal response to psychotropic agents have been studied using electrophysiological single unit recording methods. Our experiments indicate that in unanesthetized-curarized rats, intravenous haloperidol (25-200 μg/kg), L-sulpiride (20-50 mg/kg) and ethanol (0.5-2.0 g/kg) produce a dose-related increase in the firing rate of dopaminergic neurons in the pars compacta of the substantia nigra (SN-DA neurons). In such preparation, lisuride (25-50 μg/kg) produced an initial stimulation followed by a partial inhibition of firing. In rats anestetized with chloral hydrate or halothane, the stimulant effect of sulpiride and ethanol was abolished and that of haloperidol was markedly reduced. In anesthetized rats, lisuride produced a dose-related inhibition of firing without the initial stimulatory response. In unanesthetized-curarized rats, the intravenous administration of chloral hydrate (100-200 mg/kg), pentobarbital (10-40 mg/kg) and halothane inhalation (0.5 - 2.5% v/v in air) produced a dose-related increase in the firing rate of SN-DA neurons. This eventually returned to base-line in spite of the fact that the administration of the anesthetics was continued. It is concluded that in the unanesthetized paralyzed preparation, dopaminergic neurons are under tonic inhibition which is relieved by the anesthesia or by blockade of dopamine receptors.

(1) F.E. Bloom et al., J. of Pharmacol. and Exp. Therap. 150 (2), 244, 1965

293.1 KINETIC EVIDENCE FOR A MULTI-STEP PROCESS IN THE BINDING OF AN AGONIST TO BOVINE HIPPOCAMPAL SYNAPTIC MEMBRANE OPIATE RECEPTORS. S.D. Scheibe*, D.B. Bennett*, J.W. Spain*, B.L. Roth* and C.J. Coscia. E.A. Doisy Department of Biochemistry, St. Louis University School of Medicine, St. Louis, MO 63104.

Previously we demonstrated the slow dissociation of D-ala^2-D-leu^5-enkephalin (DADL), a prototypic agonist for the δ-subtype of opiate receptors, from purified bovine hippocampal synaptic plasma membranes (SPMs) (K. Pryhuber et al., Eur. J. Pharmacol. 83, 47, 1982). Current concepts of ligand-receptor interaction suggest that agonist binding is a multi-step process, whereas antagonist binding occurs by simple bimolecular association. To test this model the rates of DADL, diprenorphine and naltrexone dissociation from bovine hippocampal SPM opiate receptors were measured after varying the time of ligand association. Preliminary determination of the regulatory effects of Na^+ ion on kinetic and equilibrium binding established the agonist behavior of DADL and the antagonist properties of diprenorphine and naltrexone with these receptors. Their rates of association were also estimated. DADL exhibited a monophasic dissociation ($t_{\frac{1}{2}}$=68.9 min.; k_{-1}=1.09x10^{-2} min.$^{-1}$) after 120 min. of association (equilibrium binding). After 10 and 40 min. pre-association periods, biphasic dissociation and the following kinetic data were observed. For the fast phase of dissociation $t_{\frac{1}{2}}$=22.7 and 25.0 min. resp. and k_{-1}= 3.12x10^{-2} and 2.77x10^{-2} min.$^{-1}$ resp. ($p<0.001$ for 10 vs 120 min.); for the slow dissociation phase, $t_{\frac{1}{2}}$=112 and 101 min., resp. and k_{-1}=7.14x10^{-2} and 4.99x10^{-2} min.$^{-1}$, resp. For the antagonists, diprenorphine and naltrexone, varying association times (5, 10, 20 or 40 min.) had no effect on the dissociation rate. Computerized analysis using the weighted, least-square, non-linear regression LIGAND program revealed that DADL binding fit a one site model better than a two-site model. Parameter estimates were K_D=1.08 nM, ß=102 fmol/mg protein.

These results were consistent with the concept that in contrast to antagonists, binding of the agonist, DADL, to opiate receptors in bovine hippocampal SPM's is a multi-step process. (Supported by NSF Grant BNS-81-14947 and NIH Grant 5 T32 H1 07050-08.)

293.2 CYCLIC BIS-PENICILLAMINE ENKEPHALINS: HIGHLY IMPROVED SELECTIVITY TOWARD DELTA OPIOID RECEPTORS. K. Akiyama*, H. I. Mosberg*, R. Hurst*, K. W. Gee, H. I. Yamamura and V. J. Hruby*, Departments of Pharmacology and Chemistry, University of Arizona, Tucson, AZ 85724.

The endogenous opioid pentapeptides, (met^5)enkephalin and (leu^5)enkephalin have been shown to interact with several types of opiate receptors which are thought to mediate different biological responses. Since the role of the specific opioid receptors has been hampered by the lack of selective ligands, we sought to design enkephalin analogs with high receptor selectivity by incorporating conformational constraints such that the resultant compound can attain the conformation required for binding and transduction at one receptor but not that required for the other receptor classes. The bis-penicillamine enkephalin analogues as shown in the table were synthesized by solid phase methods and were purified by partition and gel chromatography, using previously described protocols (Mosberg et al., BBRC 106: 506, 1982). The ability to inhibit ^{3}H-(D-ala^2, D-leu^5)enkephalin(DADLE) was investigated in a proposed opioid (delta) specific neuroblastoma-glioma hybrid culture cell line. NG 108-15 cells (generously provided by D.C. U'Prichard) of low passage were grown in 90% DMEM supplemented with 10% FBS and antibiotics in an atmosphere of 90% air and 10% CO_2 at 37°C. Cells at confluency were harvested and frozen in liquid nitrogen at -80°C until used. For the radioligand binding assay, cells were thawed, homogenized, centrifuged and the pellet resuspended in 100 mM NaCl and preincubated for 60 min at 25°C. Subsequently the cell membranes were washed with 50 mM Tris-HCl buffer 3 times. Cell membranes (2 x 10^6 cells) were incubated with 0.3 nM ^{3}H-DADLE (S.A. 43.6 Ci/mmole) for 60 min at 25°C. Met-enkephalin (1 µM) was used for the determination of nonspecific binding.

Enkephalin Analogs	IC_{50}(nM)	Hill Value
(D-Pen^2, D-Pen^5)Enkephalin	3.77	1.11
(D-Pen^2, L-Pen^5)Enkephalin	3.72	1.04

The results summarized in the table illustrate that the bis-penicilliamine enkephalin analogs are potent agonist ligands and bind to a homogeneous delta receptor population (Hill slope ≈1). When these analogs were examined at mu opioid sites using ^{3}H-naloxone in rat brain homogenates, we found IC_{50} values in the 3000 nM range. Thus, the extremely high delta receptor selectivity of these compounds indicates that conformational restrictions imposed by cyclization of peptide structures provide a viable approach toward receptor selective compounds.

Supported by USPHS grants and RCDA to H. I. Yamamura.

293.3 REGIONAL SATURATION STUDIES OF 3H-NALOXONE BINDING IN RAT BRAIN. WA Geary and GF Wooten, Dept of Pharmacology, Wash.Univ. Med. School, St.Louis, MO and Dept of Neurology, Univ. of VA. Med. School, Charlottesville, VA.

The saturation characteristics (K_D and Bmax) of ^{3}H-naloxone binding in 13 rat brain nuclei were examined using quantitative film autoradiography (Geary and Wooten, JPET 225:234-240,1983). Binding studies were conducted on 10µm serial sections at room temperature (20-22°C) in phosphate buffered saline. Total binding was derived from tissue incubations with 0.75-7.0nM ^{3}H-naloxone (Amersham;45-55 Ci/mmole). Nonspecific binding was determined by incubation of adjacent sections with radioligand plus 300nM levorphanol. Raw data were analyzed according to a regression model for single phase binding interactions (Zivin and Waud, Life Sci. 30: 1407-1422,1982); all data exhibited bias error terms ≤10%. Regional kinetics values are given in the table below:

Structure	K_D ± SD (nM)	Bmax ± SD (fm/mg wt wgt)	(n)
N. accumbens (rostral)	1.87 ± 0.47	42.4 ± 7.0	5
N. accumbens (caudal)	2.06 ± 0.53	47.3 ± 5.9	5
Noncluster striatum	1.77 ± 0.17	29.6 ± 5.5	5
Whole striatum	3.19 ± 0.26	57.3 ± 7.2	5
Central anter. amygdala	1.59 ± 0.46	106.3 ± 13.5	4
Cortical amygdaloid n.	2.61 ± 0.69	102.5 ± 24.0	4
Hippocampus CA_1	0.65 ± 0.15	34.5 ± 7.4	5
Cingulate cortex	1.44 ± 0.15	37.6 ± 1.4	4
Medial-frontal cortex	1.37 ± 0.40	35.8 ± 6.4	5
Habenula	2.51 ± 0.53	152.1 ± 25.3	4
Interpeduncular n.(dorsal)	0.74 ± 0.14	142.9 ± 18.3	5
Super.coll.(superfic. gray)	1.36 ± 0.27	35.3 ± 5.5	5
Median raphé	2.70 ± 0.63	80.0 ± 10.6	5

The range of K_D values was 0.65nM (CA_1) to 3.19nM (whole striatum); ANOVA tests for heterogeneity of K_D's were significant at $p<.001$. The range of Bmax values was 29.6fm/mg (noncluster striatum) to 152.1fm/mg (habenula). The mean ± SD for K_D and Bmax for all structures pooled were 1.87 ± 0.87nM and 74.5 ± 44.5fm/mg, respectively; these values are similar to those obtained in homogenate preparations of whole brain minus cerebellum (Pert and Snyder, Mol. Pharm.10:868-879, 1974). Our results demonstrate small but definite regional variations in binding affinity for naloxone in rat brain. These regional variations in K_D may reflect different relative concentrations of opiate receptor subtypes, variation in receptor-associated lipid composition, regional variations in concentrations of endogenous opioids, and/or true structural heterogeneity in the mu_1 opiate receptor. These data will allow the calculation of regional fractional occupancy of opiate receptors with in vivo experimental paradigms.

293.4 CHARACTERIZATION OF BOVINE ADRENOMEDULLARY OPIATE RECEPTORS. M. Dumont* and S. Lemaire (Spon:E.R.-Moliner). Département de Pharmacologie, Centre Hospitalier Universitaire, Sherbrooke, Québec, Canada J1H 5N4.

The presence of high affinity stereospecific opiate binding sites in membrane preparations of bovine adrenal medulla has already been demonstrated but its identification (µ,κ,δ,σ) as well as its potency to bind some particular endogenous opioid peptide(s) remain to be determined. Using specific ligands for each type of opiate receptors as well as peptides derived from each class of endogenous opioid precursors (ß-endorphin, enkephalins, dynorphin), we have undertaken to characterize the adrenomedullary opiate receptor. Binding assays were performed at 37°C for 30 min with 2 ml-aliquots of the membrane preparation (ca. 1 mg wet weight) and 1 nM [^{3}H]-etorphine ([^{3}H]-ET). Bacitracin (2.5 x 10-5 M) was added to the incubation medium to protect the peptides from enzymatic degradation. The bound ligand was separated from free by filtration through GF/B whatman filters. Relative potencies of various opiate ligands were estimated by their ability to compete with [^{3}H]-ET for opiate receptors on the membrane preparation of bovine adrenal medulla. The opiate binding studies showed that the maximal density of binding sites (Bmax) for [^{3}H]-ET was 116 fmol/mg of protein and the half saturation (K_D) was obtained at 0.96 nM. Levorphanol was 1691 times as potent as the inactive enantiomer dextrorphan in inhibiting the binding of [^{3}H]-ET. The ability of various opiate ligands to displace the binding of [^{3}H]-ET indicates a high potency for compounds specific to the κ and µ receptors (MR2034(κ) > dihydromorphinone(µ) > ethylketocyclazocine(κ) > SKF10047 (σ) >> [D-Ala^2,D-Leu^5]-enkephalin(δ)). The experiments with the endogenous opioid ligands revealed that the opiate receptor is ß-endorphin specific, its IC_{50} being 10.5 nM as compared with 680 and 1225 nM for dynorphin and [D-Ala^2,D-Leu^5]-enkephalin, respectively. These results suggest that although the adrenomedullary opiate receptor can bind both κ and µ opiate ligands the only endogenous opioid peptide which shows high affinity for this receptor is ß-endorphin, a peptide which is not contained by the gland itself but may rise into the circulation of animals exposed to stress or some other stimuli.

(Supported by the Medical Research Council of Canada (PG-20). M.D. is a recipient of F.R.S.Q. studentship.

293.5 STRUCTURE-ACTIVITY RELATIONSHIPS OF DYN-(1-13) AND ALA-CONTAINING ANALOGS. Simon Lemaire, Andrée Turcotte* and Jean-Marc Lalonde*. Département de Pharmacologie, Centre Hospitalier Universitaire, Sherbrooke, Québec, Canada J1H 5N4.

Dyn-(1-13) and its analogs substituted by single introduction of Ala in its positions 1 to 11 were synthesized by the solid-phase procedure and analyzed for their activity on the guinea pig ileum (GPI) and the mouse vas deferens (MVD) assays as well as their ability to displace the binding of [^{3}H]-etorphine to rat brain homogenates. The synthetic compounds were found homogeneous by their migration on thin layer chromatography, their elution profiles on high pressure liquid chromatography and their amino acid compositions after acid digestion. Substitutions of positions 1, 2 and 4 by Ala caused the most dramatic decreases in the biological activity of the molecule on the smooth muscle preparations (relative potencies < 0.9%). Conversely, in the opiate binding test, the integrity of positions 1, 4 and 5 (relative potencies < 6%) was more important than that of positions 2 and 3 (relative potencies > 12%). The other substitutions which lowered much the potency of the molecule were seen in positions 6, 7, 9 and 11, four basic residues. Among these, Arg^6 and Arg^7 were demonstrated to be the most important in the three biological tests. Finally, the replacement of Ile^8 by Ala increased the relative potency of the molecule by a factor of 1.9 on the MVD and 9.0 in the opiate binding test. These results indicate that, besides the residues located inside the Leu-enkephalin core, the acidic residues of Dyn-(1-13) fulfill an important role in the particular potency of the molecule whereas Ile^8 is a position which can be replaced to give a compound with higher potency.

(Supported by the Medical Research Council of Canada and "Le Conseil de Recherches en Sciences Naturelles et Génie".

293.6 OPIOID RECEPTOR BINDING IN DEVELOPING SHEEP. C. E. Dunlap III* and J. C. Rose* (SPON: J. G. McCormick). Dept. of Physiol. & Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103.

Experiments were performed to characterize mu- and delta-type opioid receptor binding in both maternal and developing fetal sheep. Binding of 1 nM (^{3}H) dihydromorphine (DHM) and 1 nM (^{3}H) D-ala^2-D-leu^5-enkephalin (DADLE) was quantitated in homogenates of specific brain regions in sodium free 50 mM phosphate buffer. Binding levels of 1 nM DHM in maternal hippocampus, frontal cortex, and cerebellum were 4.7 ± 0.8, 10.6 ± 1.6, and 32.1 ± 2.2 fmoles/mg protein, and levels of 1 nM DADLE binding were 18.6 ± 1.5, 36.1 ± 4.0, and 19.9 ± 2.9 fmoles/mg protein, respectively (sample size varied from 3 to 8).

In developing fetal frontal cortex, both DHM and DADLE binding remained fairly constant over the gestational ages studied. DHM and DADLE binding levels in mid-gestation, 77 day fetal frontal cortex were 12.5 and 17.0 fmoles/mg protein, respectively, compared to 12.1 and 14.8 fmoles/mg protein in the newborn. In hippocampus, on the other hand, increases in both DHM and DADLE binding were noted over the course of gestation. Binding increased from 2.6 fmoles/mg protein for DHM and 9.7 fmoles/mg protein for DADLE in 70 day hippocampal tissue to 5.0 fmoles/mg protein for DHM and 14.2 fmoles/mg protein for DADLE in hippocampus from newborn sheep. The same case was seen for DHM binding in cerebellum, increasing from 21.1 to 31.8 fmoles/mg protein in 77 and 108 day fetuses, respectively. However, DADLE binding was observed to remain fairly constant in cerebellum during gestation, with a value of 18.8 fmoles/mg protein at 77 days gestation compared to 18.6 fmoles/mg protein at 108 days gestation.

The reasons for differential development of mu- and delta-opioid receptors observed during gestation in sheep are not known but are currently being investigated in our laboratory. It is also of interest to note the high levels of both mu- and delta-receptor binding in sheep cerebellum as compared to other species, especially rodents.

This research was supported by NIH grant HD 11210.

293.7 DIFFERENTIATION OF MU, KAPPA AND DELTA SELECTIVE LIGANDS FOR OPIOID RECEPTORS IN GUINEA PIG BRAIN Richard A. Ferrari*, Teresa K. Ramos*, Concetta Zobre*, and Susan J. Ward*. (SPON.: J.K. Saelens). Dept. of Pharmacology, Sterling-Winthrop Research Inst., Rensselaer, New York 12144.

Guinea pig brain contains mu, kappa, and delta receptors (Kosterlitz, Paterson, and Robson, Brit. J. Pharmacol. 73, 939, 1981). ^{3}H-Etorphine binds to these three receptor subtypes (Tolkovsky, Mol. Pharmacol. 22, 648, 1982) and may be more universal than most opioid ligands. We investigated the possibility of masking one or two of these receptor populations with selective, unlabeled ligands in order to define receptor affinity of various opioids.

Guinea pig brains, minus cerebellum, were homogenized in pH 7.7 Tris buffer, centrifuged, resuspended in Tris buffer and incubated at 37^{0}C to degrade endogenous opioids. The suspension of brain membranes was stored at -20^{0}C prior to its use within one week. A Scatchard plot with high specific activity ^{3}H-etorphine (45 Ci/mmole) gave a K_D of 0.09 nM for the high affinity site using 10 uM naloxone to determine non-specific binding.

U-50,488, a kappa selective agonist, gave a K_I of 490 nM against 0.09 nM ^{3}H-etorphine. However, when 50 nM each of D-Ala^2, D-Leu^5-enkephalin (DADLE, which binds selectively to delta receptors) and Tyr-D-Ala-Gly-Me-Phe-NH$(CH_2)_2$NO$(CH_3)_2$ (RX-783030, which binds selectively to mu receptors) was added to the above system, U-50,488 bound with an affinity of about 200-fold greater than when the mu and delta receptors were not masked. When D-Ser^2,Thr^6-leuenkephalin (DSLET, which is selective for delta receptors) was tested in the presence of the DADLE and RX-783030, very little binding of DSLET occurred, confirming that the delta sites were masked. The K_I for normorphine, on the other hand, was 20 nM in the absence of and 100 nM in the presence of DADLE and RX-783030. Binding of 100 nM U-50,488 incubated with 50 nM DADLE or with 50 nM RX-783030 was enhanced 93 and 66% respectively compared with 144% when incubated all together, indicating an additive effect of these compounds. Masking experiments of this type may be used to differentiate receptor subtypes.

293.8 BIOCHEMICAL EVIDENCE FOR MU_1 SITES: A COMMON, VERY HIGH AFFINITY BINDING SITE FOR OPIATES AND ENKEPHALINS. S.L. Nishimura*, L. Recht and G.W. Pasternak (SPON: J.B. Posner). Departments of Neurology and Pharmacology, Memorial Sloan-Kettering Cancer Center and Cornell University Medical College, New York, N. Y. 10021 USA.

Previous studies have suggested a common high affinity (K_D <1 nM) binding component for opiates and enkephalins, termed mu_1 as well as selective sites for the various classes of opioids with slightly lower affinity (mu_2, delta, kappa, and sigma; K_D 2-15 nM). Saturation studies performed with either ^{3}H-dihydromorphine in the presence of unlabeled D-ala^2-D-leu^5-enkephalin (1 nM) or ^{3}H-D-ala^2-D-leu^5-enkephalin in the presence of unlabeled morphine (1 nM), demonstrated low concentrations of both unlabeled opioids more potenty inhibited the high affinity binding of their complimentary radiolabeled ligand, consistent with a common high affinity site for opiates and enkephalins. Treatment of tissue with N-ethylmaleimide (25 μM) effectively abolished the high binding component of both ^{3}H-dihydromorphine and ^{3}H-D-ala^2-D-leu^5-enkephalin. The lower affinity component of ^{3}H-dihydromorphine was unaffected while that of ^{3}H-D-ala^2-D-leu^5-enkephalin was decreased only 35%. Protection experiments examining N-ethylmaleimide (25 μM) inhibition of ^{3}H-dihydromorphine binding showed significant protection ($p<0.002$) by both unlabeled D-ala^2-D-leu^5-enkephalin and morphine (both at 1 nM). When studied together, both naloxonazine and N-ethylmaleimide inhibited ^{3}H-dihydromorphine binding to a similar extent. Equally important, tissue treated with naloxonazine was far less sensitive to N-ethylmaleimide than untreated control tissue, consistent with the possibility that both treatments affected the same site. Together, these results support the concept of a common high affinity binding site for morphine and the enkephalins.

293.9 ONTOGENY OF MULTIPLE OPIATE RECEPTORS (μ, δ, κ): A COMPUTERIZED ANALYSIS. J.W. Spain*, D.B. Bennett*, B.L. Roth* and C.J. Coscia (SPON: K. Smith). E.A. Doisy Department of Biochemistry, St. Louis University School of Medicine, St. Louis, MO 63104.

In a continuing investigation of the postnatal development of opiate receptor subtypes in rat brain, we compared the appearance of benzomorphan-selective sites with μ and δ receptors. The affinity and capacity of 0.5 nM [^{3}H]-ethylketocyclazocine (EKC) binding in 50 mM Tris (pH 7.4) was measured using crude brain homogenates by displacement with unlabeled EKC, morphine and D-ala^2-D-leu^5-enkephalin (DADL). Binding curves were simultaneously analyzed using the weighted, least-squares, non-linear regression, curve-fitting LIGAND program (Munson and Rodbard, 1980). Parameter estimates were obtained for rats at days 1, 5, 7, 11, 14, 21 after birth.

At all stages of development, EKC binding fit a multiple site model significantly better than a one site model. Neither high nor low EKC binding affinity varied significantly in the postnatal period, e.g., the apparent K_D's for 1 day old rats were 0.92 and 20.3 nM whereas for adults they were 0.97 and 17.7 nM, respectively. The affinities of morphine and DADL for the EKC binding site did not change in this period. A 20-fold elevation in EKC specific binding per brain was observed from birth to maturity. The appearance of high affinity κ receptors during the post-natal period showed a linear 14-fold increase (pmol/brain) paralleling that of the μ subtype and different from that of the δ sites. The number of low affinity κ receptors remained unchanged through the first week and increased 8-fold during the following two weeks in a manner similar to that observed for the δ site. However, the K_D for DADL at the low affinity EKC binding site is 20 μM.

Subcellular fractions from adult rat forebrain were also analyzed for naloxone, DADL and EKC selective sites by displacement analysis. The results indicated that μ, δ and κ sites were enriched in both synaptic membranes and microsomes. Naloxone and EKC binding fit a multiple site model for both subcellular populations as did DADL in microsomes. However, parameter estimates for DADL binding to synaptic membranes were more consistent with a single binding site.

In conclusion, the results suggest the existence of a discrete population of high affinity EKC binding sites which are present at birth in rat brain and increase linearly during maturation.
(Supported by NSF Grant 81-14947 and NIH Grant 5 T32 HL 07050-08.)

293.10 EFFECTS OF SPECIFIC OPIATE AGONISTS IN THE CONDITIONED TASTE AVERSION. M. Ng Cheong Ton* & Z. Amit, (SPON: R. E. Musty). Ctr. for Behav. Neurobiol., Concordia University, Rm 1013, 1455 de Maisonneuve Blvd., Montreal, Que., Canada H3G 1M7

There is accumulating evidence that there are different populations of opiate receptors (Wood, Charleson, Lane & Hudgin, 1981) in the CNS. Apparently each population of opiate receptors serves a very specific and selective function. We have conjectured that a specific population of opiate receptors is responsible for the induction of the conditioned taste aversion (CTA) by opiates. As a preliminary work to test this hypothesis, we have selected three different specific agonists namely morphine(M), ethylketocyclazocine(EKC), and SKF-10047(SKF) and investigated their profile actions in the CTA paradigm. Rats were randomly assigned to one of the four experimental groups after a week of adaptation to a 30-minute daily access to water schedule. On Days 1 and 6, animals received an injection of either M (12 mg/kg) or EKC (6 mg/kg) or SKF (6 mg/kg) or vehicle i.p. immediately following a 30-minute access to 0.1% saccharin solution. Vehicle consisted of a mixture of 8.5% lactic acid and 1.0 N sodium hydroxide in a 3:2 ratio. On Days 11 and 16, animals were only presented with saccharin without drug injection. Using the Dunnett's test, it is found that the vehicle group showed increased preference for saccharin at all trials. The M and SKF groups both showed an aversion for saccharin on the two pairing days. The EKC group interestingly did not show a substantial decrease in saccharin consumption on the first trial but only on the second trial. In addition, the EKC group showed a faster recovery during the extinction trials; there was a significant increase in saccharin intake on the last day. Furthermore, using the LSD test, it is found that the decrease in saccharin intake in the SKF group on the second trial was significant greater than the decrease observed in either the M and EKC groups on the same trial. These results suggest that to the extent that those drugs are rather selective agonists for specific types of opiate receptors, the sigma and the mu receptors seem to be more important than the kappa receptors in the mediation of CTA produced by opiates.

293.11 SELECTIVE ALTERATIONS IN GUANINE NUCLEOTIDE REGULATION OF OPIATE RECEPTOR BINDING AND COUPLING WITH ADENYLATE CYCLASE. Steven R. Childers, Scott M. Lambert*, and Geertje La Riviere*. Department of Pharmacology, University of Florida College of Medicine, Gainesville, FL 32610

GTP couples receptors with adenylate cyclase and increases dissociation of agonists at receptor binding sites. We have previously shown that GTP effects on binding were selectively lost when membranes were incubated with N-ethylmaleimide in the presence of opiate ligands. We now report that GTP effects on opiate agonist binding are selectively increased by pretreatment of membranes at low pH, conditions which eliminate stimulatory GTP coupling to cyclase.

Rat brain membranes were incubated with 50 mM Na acetate, pH 4.5, at 25° for 20 min, centrifuged and resuspended in 50 mM Tris, pH 7.7 for radioreceptor assay. Results showed that low pH pretreatment had no effect on binding of ^{3}H-agonists alone, but that the maximum effect of GTP and Gpp(NH)p on inhibiting ^{3}H-agonist binding was dramatically increased. For ^{3}H-D-ala enk binding, the maximal GTP effect was increased from 35% to 80% inhibition of binding by low pH pretreatment.

To compare the effects of low pH on GTP regulation of binding and on coupling to cyclase, we assayed adenylate cyclase in membranes pretreated at pH 4.5, then resuspended in cyclase buffer, pH 7.4. Loss of basal cyclase activity in rat brain membranes, as seen both in the absence of stimulatory agents and in the presence of 10 mM Mn^{2+} to stimulate catalytic units, was minimal after low pH pretreatment. Surprisingly, GTP-stimulated activity, as measured by stimulation with 10 mM NaF or 10 μM Gpp(NH)p, was severely reduced (by 60-95%), indicating a functional loss of stimulatory GTP-coupling proteins. In rat striatal membranes, dopamine-stimulated cyclase was also severely reduced, although basal and Mn^{2+}-stimulated activity was not affected. The effect of low pH pretreatment on cyclase was reversed by incubating membranes after low pH treatment with fluidizing agents (cis-vaccenic acid and phosphatidyl choline) which restored NaF- and Gpp(NH)p-stimulated activity. However, the increase in the GTP-induced inhibition of agonist binding caused by low pH was not reversed by fluidizing agents. These results suggest that the GTP-binding proteins which regulate opiate receptor binding and those which stimulate adenylate cyclase are fundamentally different.

Supported by PHS grant DA02904.

293.12 MECHANISMS OF PHOSPHOLIPASE A_2-INDUCED BEHAVIORAL AND CHEMICAL EFFECTS IN RAT PERIAQUADUCTAL GRAY MATTER. M. Reichman*, L.G. Abood, and M. Costanza* Center for Brain Research, University of Rochester School of Medicine, Rochester, New York 14642.

To understand the functional role of the opioid receptor in the rat's periaquaductal (PAG) as well as the possible role of phospholipids, 0.2 nmole bee venom phospholipase A_2 (PLA_2) was administered into the PAG in awake rats, through chronically implanted cannulae. Within 2 hr, the rats exhibited intermittent explosive motor behavior but no analgesia as measured by tail flick. Neither trypsin nor 1 umole lysophosphatidyl choline (LPC) resulted in a similar effect. LPC did not produce analgesia nor did it attenuate morphine induced analgesia. In another set of experiments, after PLA_2 was injected into the PAG of rats under barbital anesthesia, the PAG was rapidly removed and analyzed for free fatty acids (FA) or ^{3}H-dihydromorphine (^{3}H-DHM) binding. Unsaturated FA increased 3-fold over the controls while saturated FA were unchanged. ^{3}H-DHM binding decreased 70% in the PLA_2-treated rats. We conclude: 1) that the behavioral effects of PLA_2 into the PAG results from the unique lipolytic activity of the enzyme rather than generalized membrane damage; and 2) the opioid sites for analgesia in the PAG are distinct from those associated with explosive motor behavior. Supported by DA 00464.

293.13 AUTORADIOGRAPHIC LOCALIZATION OF MU OPIATE BINDING SITES IN HIPPOCAMPUS. B. J. Crain, K.-J. Chang, and J. O. McNamara. Departments of Medicine (Neurology), Pathology, and Pharmacology, Duke University; Epilepsy Centers, Duke University and VA Medical Centers, and The Wellcome Research Laboratories, Research Triangle Park, Durham, North Carolina 27710.

One approach to the problem of determining the functions of endogenous opiate ligands in normal hippocampal formation is to determine the anatomic distributions of the various subpopulations of opiate binding sites. Thus, we have examined in detail the laminar distribution of mu opiate binding sites labeled with [^{125}I]-FK-33824 in normal adult rat hippocampus.

Slide-mounted sections were preincubated in 50 mM Tris-HCl, pH 7.7, with 100 mM NaCl and 0.1 mM GDP to remove endogenous ligands and then incubated in 0.7 nM [^{125}I]-FK-33824 in 170 mM Tris-HCl, pH 7.7, and 5 mM $MgCl_2$ at 25° C for 60 minutes. Labeled sections were apposed to [^{3}H]-Ultrofilm or emulsion-coated coverslips (Kodak NTB-2) for one to six weeks.

In the hippocampus, the most intense specific labeling by [^{125}I]-FK-33824 occurred in a band over stratum pyramidale which extended slightly over the immediately adjacent portions of stratum oriens and stratum radiatum. Binding in this band was slightly greater in CA3 than in CA1. Labeling of similar intensity was present in a club-shaped area of stratum lacunosum-moleculare adjacent to the end of the hippocampal fissure and slightly less labeling was present in the deeper half of stratum lacunosum-moleculare in CA1 and CA3 adjacent to stratum radiatum. In contrast, the superficial half of stratum lacunosum-moleculare in CA1 and CA3, all of stratum radiatum in CA1, and stratum lucidum in CA3 were only very lightly labeled. Intermediate levels of [^{125}I]-FK-33824 binding were present in stratum radiatum and stratum oriens of CA3 and in the outer part of stratum oriens of CA1, as well as in stratum moleculare and in the hilus of the dentate gyrus. Binding was slightly increased over the outer third of stratum moleculare compared to the inner two-thirds. Very little labeling was present over stratum granulosum.

Based on these results, the laminar distribution of the mu subpopulation of opiate binding sites appears to correlate with the distribution of hippocampal interneurons rather than with the distributions of known afferents. This pattern suggests that mu opiate ligands may influence hippocampal neuronal activity through effects on multiple populations of interneurons.

293.14 PHARMACOLOGICAL CHARACTERISTICS AND AUTORADIOGRAPHIC DISTRIBUTION OF [^{3}H]-EKC OPIATE BINDING SITES IN RAT AND GUINEA PIG BRAIN. A.S. Weiss*, C.B. Pert, and R. Quirion (SPON: J.T. Molt). Section on Brain Biochem, NSB, NIMH, Bethesda MD 20205 and Dept of Physiol Biophys, Hahnemann Univ, Philadelphia PA 19102.

We recently reported that the autoradiographic distribution of [^{3}H]ethylketocyclazocine (EKC) binding sites, a prototype kappa agonist, is very similar to those of μ opiate receptors in rat brain (Quirion et al., Cell.Mol.Neurobiol., 2:333, 1982). We now report further characterization of [^{3}H]EKC binding in rat and guinea pig brain using an autoradiographic technique (Herkenham and Pert, J.Neurosci. 2:1129, 1982).

Slide-mounted brain sections were preincubated in 50 mM K_2HPO_4-HCl buffer, pH 7.4 at 4°C (or 25°C) for 15 min, then incubated in the same buffer plus 100 nM morphiceptin, 100 nM D-Ser^2-Leu^5-Thr^6-enkephalin, 0.5 mg/ml bacitracin, 0.1% BSA, and 2.8 nM [^{3}H]-EKC at 25°C (or 4°C) for one hr with several concentrations of various opioids and opiates. After incubation, slides were transferred through 4 4-min cold buffer washes. Binding of [^{3}H]-EKC to the brain slice was quantitated by assaying the tissue-bearing slide fragment in 10 ml Aquassure scintillation cocktail. Specific binding was determined by the difference in [^{3}H]-EKC bound in the presence and absence of 1 μM (-) bremazocine. Autoradiographic studies followed similar procedures through the wash step. Slides were then air-dried rapidly and placed tightly in film cassettes containing tritium-sensitive film at room temp for six wks. Visualization of kappa receptors and quantification of optical density data were determined by computerized densitometry. Saturation curves and Scatchard analysis show that [^{3}H]-EKC binds to an apparent single saturable class of receptors in guinea pig brain. The K_D was 1.53 nM and the B_{max} was 31.55 fmole/slice under mu and delta suppression at 25°C. The K_D was 2.69 nM and the B_{max} was 63.56 fmole/slice with 100 mM NaCl in the incubation medium at 4°C. Ligand selectivity studies demonstrate that several opioids and opiates are potent displacers of [^{3}H]-EKC binding. The relative order of potency (IC_{50}, nM) is: (-) Bremazocine (4.3) > Dynorphin B (5.5) > Dynorphin 1-13 (6.1) > Dynorphin 1-9 (6.2) > Alpha-neo-endorphin (7.0) > Dynorphin 1-17 (9.4) > MR 2034 (10.1) > MR 2266 (11.3) > Peptide E (11.7) > BAM-12P (18.8) > BAM-22P (20.0) > Dynorphin 1-8 (38.2) >> Beta-endorphin (265) >> Leu^5-enkephalin (>2000) = Met^5-enkephalin (>2000). These results strongly suggest a kappa-like selectivity pattern. Autoradiographic studies show high levels of kappa receptors in the substantia nigra, thalamus, nucleus accumbens, superior and inferior colliculi, olfactory bulb, amygdala, cerebral cortex, IP nucleus, and the PAG in both rat and guinea pig brain. Cortical distribution in the rat was uniform while kappa receptors are primarily in layers 5 and 6 of guinea pig cortex.

293.15 EFFECTS OF NUCLEOTIDES ON ETHYLKETOCYCLAZOCINE BINDING. K. J. Mack, M. Butterfield*, M. Butterfield*, A. Killian* and J. A. Weyhenmeyer. College of Medicine, University of Illinois, Urbana, IL 61801.

Recent studies on the effects of guanyl nucleotides on mu and delta agonist binding have shown decreases in total binding of opiates to brain membranes in the presence of GTP and GPP(NH)P. In this study, we report the influence of nucleotides on the binding of the prototypic kappa ligand, ethylketocyclazocine (EKC), to rat brain membranes.

Brain membrane homogenates were incubated with 1 nM ^{3}H-EKC in Tris, pH 7.4, for 60 min at 25°C. The binding of ^{3}H-EKC was not affected by additions of 0.3 to 1000 μM ATP, CTP, UTP, GDP or GMP. However, the addition of GTP (IC_{50}, 178 μM) or GPP(NH)P (IC_{50}, 624 μM) resulted in decreased binding of the kappa ligand.

Scatchard analysis was used to determine whether the observed decrease in binding was due to changes in either affinity or binding capacity. In control binding assays (in the absence of nucleotides), Scatchard analysis revealed linear plots with an affinity (K_d) of 0.92 nM and maximum binding capacity (B_{max}) of 260 fm/mg. Semilogarithmic plots (bound vs. log free concentration) were used to demonstrate that the curve passed the inflection point and approached a plateau. The addition of 0.1 to 1.0 mM GTP to the incubation medium resulted in a curvilinear plot with a high affinity site of less than 0.3 nM and a low affinity site of greater than 6 nM. The maximum binding capacity either increased or decreased in relation to the concentration of GTP. Approximately 5% of the total binding sites were attributable to the high affinity site. Under conditions of 0.01 to 1 mM GPP(NH)P, linear plots were observed with changes in binding capacity.

This study provides evidence to suggest that GTP affects both the affinity and binding capacity of the prototypic kappa ligand, ethylketocyclazocine. Further, these effects appear to be dependent on both the concentration of the nucleotide and the isotope.

This work was supported by USPH grant HL 27757 to J.A.W. K.J.M. is supported by a predoctoral fellowship on USPHS grant GM 07143.

293.16 IN VITRO INTERACTIONS BETWEEN FENTANYL AND BUPRENORPHINE. J.W. Villiger and R.A. Boas, Department of Pharmacology and Clinical Pharmacology, University of Auckland School of Medicine, Auckland, New Zealand.

We are presently studying the ability of the partial opiate agonist buprenorphine, administered toward the end of high dose fentanyl anaesthesia, to maintain postoperative analgesia without significant respiratory depression. Since the molecular mechanisms which govern the interactions between these opiates are not fully understood, we have conducted a series of in vitro studies examining the interactions between fentanyl and buprenorphine at opiate receptors in homogenates of rat brain using a conventional filtration [^{3}H]ligand binding assay (Villiger and Taylor, J. Neurochem. 38, 1771-1773, 1982). An initial determination of the relative affinities of fentanyl and buprenorphine for the sites labelled by [^{3}H]fentanyl (2nM) yielded IC_{50} values of 0.6nM for buprenorphine and 1.4nM for fentanyl. Given clinical observations that the onset and termination of fentanyl action is more rapid than that of buprenorphine, we also determined the rates of association and dissociation for these drugs. [^{3}H]-Fentanyl binding was characterized by a comparatively rapid association with the receptor with equilibrium being reached by 10 min, whereas [^{3}H]buprenorphine (1nM) binding was a slower process, taking 30 min to reach equilibrium. [^{3}H]Fentanyl dissociation from the receptor was more rapid (initial $t_{½}$ = 4min) than [^{3}H]-buprenorphine dissociation (initial $t_{½}$ = 40min). The dissociation of [^{3}H]fentanyl binding induced by buprenorphine was both biphasic and depended on buprenorphine concentration. In the presence of 1nM buprenorphine the $t_{½}$ for the rapid dissociation phase was 25 min, the $t_{½}$ for the slower phase was > 240 min with 50% of the initial [^{3}H]fentanyl still bound after 2 hrs. In the presence of 2nM buprenorphine the respective values were 25 min, 170 min and 35%. We then examined the binding of [^{3}H]fentanyl to membranes which had previously been equilibrated with 0.5, 1.0 and 2.0nM buprenorphine. The percentages of control [^{3}H]fentanyl binding were: 37% with 0.5nM buprenorphine, 11% after 1nM and 3% following 2nM buprenorphine. These studies indicate that: 1) buprenorphine has 2.3 times greater affinity for the μ opiate receptor than fentanyl, 2) fentanyl association and dissociation is more rapid than that of buprenorphine, 3) fentanyl dissociation from the receptor is biphasic, 4) 65% of fentanyl is displaced from the receptor following addition of an equimolar concentration of buprenorphine and 5) pre-equilibrating membranes with buprenorphine is capable of almost completely inhibiting the binding of an equimolar concentration of fentanyl. These findings may help explain analgesic and respiratory responses seen in man following co-administration of fentanyl and buprenorphine.

294.1 POSTNATAL MATURATION OF THE LHRH SYSTEM. S. Wray and G.E. Hoffman. Department of Anatomy, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642.

This study immunocytochemically examined the postnatal development of the luteinizing hormone-releasing hormone (LHRH) system in both male and female rats. All animals were perfused with a neutral picric acid formalin fixative. The brains were removed and postfixed in this same fixative for 2-12 hours. The tissue was blocked and then sectioned at 50 microns using a vibrating microtome. Consecutive sections were taken from the level of the olfactory peduncles caudally through the median eminence. At least one male and female rat were processed simultaneously to allow comparisons in staining intensities to be made. The animals were coded so that the sex and age were unknown when examined at the light microscopic level. Each section was scanned at 20-40X and immunoreactive cells counted. A portion of the cell soma had to be present for the cell to be scored within that particular tissue section.

The regional distribution of LHRH did not vary with age in male and female animals. In all ages examined the majority of LHRH perikarya were present within an inverted V-shaped field. The apex of this field lay in the preoptic/septal area and extended caudally to the ventrolateral anterior hypothalamus. The total cell number visualized also did not vary over age in either male or female animals. LHRH total cell number for all animals examined was 1380 ± 35 ($\bar{x}$ ± S.E., with males being slightly lower 1327 ± 41 than females 1434 ± 57).

To determine if a 'silent' population of LHRH cells existed which were not being detected immunocytochemically, colchicine was administered to males and females at various ages. No changes in the regional distribution of LHRH occurred in either male or female animals after colchicine administration. However, an increase in total cell number was noted in both sexes. Approximately 300 additional LHRH cells were revealed after colchicine treatment (1672 ± 115). The reason these LHRH cells were immunonegative under noncolchicine conditions is unclear. Perhaps these cells contained insufficient peptide due to the physiological status of the animal. Whether the number of these 'silent' LHRH cells, and thus the total LHRH population, changes over development in male and female animals remains to be determined.

Supported by NIH Grant NS13725 and MH 08838

294.2 ANGIOTENSIN I-LIKE IMMUNOREACTIVITY IN NEPHRECTOMIZED RAT CENTRAL NERVOUS SYSTEM. D.P.Healy* and M.P. Printz*. (SPON: K. Beaumont) Division of Pharmacology M013H, Univ. Calif. San Diego, La Jolla CA. 92093.

A great deal of evidence indicates that a complete angiotensin II (ANG II) system exists in the central nervous system (CNS) as all the components of a biosynthetic pathway have been identified and appear to have an extravascular location. ANG II has been localized intraneuronally in the CNS by immunohistochemistry which suggests an intracellular site of synthesis However a number of investigators have failed to detect the immediate precursor of ANG II, i.e., the decapeptide angiotensin I (ANG I), in the CNS by immunohistochemistry raising doubts as to whether ANG I is the precursor for the central synthesis of ANG II. We have re-examined ANG I immunohistochemical staining using the highly sensitive avidin/biotin immunoperoxidase method and have sought 1. to determine whether ANG I can be localized in the CNS and, 2. to compare the staining of ANG I with the well characterized pattern for ANG II.

Male Sprague-Dawley rats (250 gm) were nephrectomized 24 hours prior to transcardiac perfusion and sacrifice. The brains were processed by the avidin/biotin immunoperoxidase method ANG I primary antisera (1:1000 dilution), kindly provided by Dr. Detlev Ganten, University of Heidelberg, was used. Staining with ANG I antisera was blocked by preabsorption of the antisera with excess ANG I but not by excess ANG II.

Immunoreactive ANG I-like positively stained neurons were seen in the medial amygdala, perifornical area, magnocellular paraventricular n., periventricular n. and the globus pallidus. ANG I-like terminal staining was seen in the median eminence interna, organum vasculosum laminae terminalis, subfornical organ and the posterior pituitary. The staining pattern for ANG I overlapped the distribution of ANG II immunoreactive staining. Generally, areas which contained positively stained ANG II neurons also contained ANG I positive neurons, whereas the converse was not always necessarily true, e.g., the globus pallidus contained ANG I neurons but not ANG II. These results indicate that ANG I may be the precursor for ANG II (as in the peripheral circulation) in such areas (although co-localization of the peptides in the same neuron has not yet been established). However, localization of ANG I alone without coincident ANG II suggests either a rapid conversion of ANG I to ANG II or a biosynthetic pathway for ANG II which is independent of ANG I in these areas. (This work was supported by HL 25457, Hypertension SCOR.)

294.3 BRAIN SPECIFIC NEURONAL POLYPEPTIDE DEDUCED FROM mRNA CLONING: IMMUNOCYTOCHEMICAL MAPPING. E.L.F. Battenberg, F.E. Bloom, R Milner*, R. Houghten* †, and G. Sutcliffe* †. Alcohol Research Center, The Salk Institute, and †Research Institute of the Scripps Clinic, La Jolla, CA 92037.

We use recombinant DNA techniques to characterize novel brain specific proteins. From our library of cDNA clones prepared from rat brain mRNAs, we have selected and determined the 1500 nucleotide sequence of a cDNA clone (plB236) corresponding to a mRNA expressed in brain, but not in rat liver or kidney. The amino acid sequence of the deduced protein (1B236) coded by this mRNA resembles a precursor for known neuropeptides with 3 possible oligopeptides at its C-terminus separated by basic dipeptide residues. Antibodies raised against corresponding synthetic peptides generally detect a common series of widely distributed neuronal structures but show some minor regional variations. The distinctive features of this system are revealed most extensively in neocortex, hippocampus and cerebellum. Thick varicose fibers surround CA3 pyramidal neurons and all Purkinje neurons; thick apparent boutons suggest synaptic contact with these and other perikarya. In cerebral cortex, immunoreactivity is most intense within the posterior cingulate and somatosensory cortex; radially directed neurites extend across layers III, IV and V, exhibit a tangentially directed presumptive terminal field in layer I and tangential axons within the cingulum bundle. Fiber staining is also detected within heavily myelinated tracts in the fornix, striatum, and lateral olfactory tract. Immunoreactive perikarya, seen best after colchicine (60 ugm, i.c.v, 48 hrs), are also detected with antisera against all three peptides; such treatments also reduce fiber-like staining in cortical layer I and in cerebellar cortex. Such immunoreactive perikarya were observed most clearly in the medial nucleus of the trapezoid body, the ventral pontine tegmentum, the ventro-medial and basal arcuate areas of the hypothalamus, and in more widely distributed and less intensely stained neurons in several other regions. The Accessory Olfactory Nucleus exhibits extensive immunoreactivity of cells and fibers, while most regions of amygdala, frontal and rhinencephalic cortex, and mesencephalon are negative. All immunoreactivity was selectively blocked by pre-adsorption by the appropriate synthetic immunogen peptides. The specific, overlapping patterns of neuronal immunoreactivity suggest a functional role of one or more of these deduced peptides in neurotransmission, but other criteria await satisfaction. Our molecular biological identification of gene products expressed selectively in brain may provide a direct approach to rare molecules for which there are no known functional assays. (Supported by grants from McNeil Labs and the Sun Oil Foundation.)

294.4 THE ONTOGENY OF NEUROTENSIN IN THE RAT MEDIAL PREOPTIC AREA: A QUANTITATIVE IMMUNOCHEMICAL ANALYSIS. B Wolfson,* R. W. Manning,* L. G. Davis and F. Baldino, Jr., E I du Pont de Nemours & Co., CR & D Dept. Glenolden Laboratory, Glenolden, PA 19036

High concentrations of neurotensin (NT) and high-affinity binding sites for this peptide have been identified in the medial preoptic area (MPO) of the rat a region long implicated in the central control of thermoregulation. Central administration of NT produces a profound hypothermia and intolerance to cold in adult rats, but much less dramatic effects in neonatal rats. These observations suggest some component of NT-containing networks in the rat CNS mature after birth. The purpose of this study was to quantitatively investigate the post-natal ontogeny of NT in the MPO both in situ and in organotypic MPO explants prepared from newborn rats and maintained in vitro

The peroxidase anti-peroxidase method of Sternberger was used to detect NT-like immunoreactivity in tissue sections prepared from colchicine-treated Sprague-Dawley rats of various ages and in MPO explants processed as whole mounts. Levels of NT-immunoreactive substances present in unfixed MPO at various ages were quantitated by ELISA and RIA procedures The ELISA was also employed to assess the specificity of the primary antiserum (ImmunoNuclear) used in the immunohistochemical studies. HPLC and immunoassays were employed to determine the nature of the NT-immunoreactive material extracted.

On post-partum day zero, no NT-like immunoreactive neurons could be identified within the MPO. At this age, however, NT-immunoreactive cells and fibers were present in the lateral preoptic area, the bed nucleus of the stria terminalis, the septo-hypothalamic area, the amygdala and the piriform cortex NT-like immunoreactive neurons were identified in the MPO of rats 9 days post-partum or older After 9 days post-partum, the number of NT-containing cells in the MPO increased with age, as the area occupied by this nucleus expanded dorsally Likewise, the quantity of NT within the MPO, as determined by neurochemical methods, increased as a function of age. NT-containing cells were also detected in MPO explants maintained in vitro 14 days or longer even though these cultures were prepared from newborn MPO in which NT cells could not be identified. These data indicate that maturation of the MPO occurs post-natally Furthermore the temporal differentiation of NT-immunoreactive neurons in vitro parallels the ontogeny of NT in vivo and suggests that the genotype for NT is present but not expressed within the MPO at birth

294.5 DISTRIBUTION OF RANATENSIN AND BOMBESIN LIKE IMMUNOREACTIVITY IN RAT BRAIN. R.M. Knight*, B.M. Chronwall*, J.Y. Pisano*, T.W. Moody, T.L. O'Donohue (SPON: V.J. Massari). Experimental Therapeutics Branch, National Institute of Neurological and Communicative Disorders and Stroke and Laboratory of Chemistry National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20205 and Department of Biochemistry, George Washington University, Washington, D.C.

Ranatensin and bombesin were originally identified and characterized from the skin of frogs. These peptides are part of a family of related peptides which include alytesin, litorin and gastrin releasing peptide which was isolated from mammals. Immunoreactive bombesin and ranatensin have been identified previously in rat brain (Moody et al., Peptides 2:75-79, 1981; O'Donohue et al., Soc Neurosci Absts No 161.20, 1982.)

To study the distribution of ranatensin and bombesin like immunoreactivity (RT and BN ir) in the rat brain, antibodies against these peptides were raised in rabbits The indirect immunofluorescence method was used on 20 µm cryostat sections from normal and colchicine treated rats perfused with 0.5% sodium nitrite in PBS followed by 4% formaldehyde in PBS (pH 7.4). Preabsorption of RT antiserum (1:1000) with RT or BN antiserum (1:1000) with BN obliterated all staining. After preabsorption of RT antiserum with BN or BN antiserum with RT, staining ability of the antiserum was retained, demonstrating that BN and RT are distinct entities in rat brain. These results were corroborated by radioimmunoassay combined with reversed phase high pressure liquid chromatography. Results of these studies demonstrate that immunoreactive BN and RT elute differently from a µBondapak C 18 column.

The distribution of immunoreactive perikarya was limited RT and BN positive perikarya were found in stria terminalis, nucleus (n) lateralis septi, n. preopticus medialis, n. paraventricularis, and n. tractus solitarii. In addition, BNir perikarya were found in the substantia grisea centralis and RT ir perikarya in n. amygdaloideus centralis, n. arcuatus and the medial habenula. The most prominent RT ir cell group was located in the dorsal tegmental gray of the pons.

Immunoreactive fibers and varicosities also showed a restricted distribution and rarely reached densities seen with many other neuropeptides. RT and BN ir were high in nucleus amygdaloideus, medium in n. interstitialis striae terminalis, n. preopticus medialis, n. hypothalamicus anterior, n. paraventricularis, n. suprachiasmaticus, n. mamillaris and n. reticularis gigantocellularis. In addition RT ir is high in the septal area, n. arcuatus and in n. amygdaloideus posterior.

The results of this study demonstrate that two distinct neuronal systems exist, one containing irBN and one containing irRT.

294.6 MOTILIN RELATED IMMUNOREACTIVITY IN MAMMALIAN ADENOHYPOPHYSIS AND HUMAN PITUITARY ADENOMA SOMATOTROPHS. C. M. Loftus*, G. Nilaver, R. Defendini*, K. D. Post*, and M. Beinfeld. (SPON: T. Pedley) Laboratory of Neuroendocrinology, Departments of Neurosurgery, Neurology, and Neuropathology College of Physicians and Surgeons Columbia University, New York, NY 10032

Motilin a 22,000 dalton peptide first isolated from porcine gut and recently demonstrated in the brain and anterior pituitary of several mammalian species, was localized immunocytochemically in guinea pig and human anterior pituitary glands and in selected human pituitary adenomas with an antiserum to synthetic porcine motilin. Deparaffinized sections incubated with the specific antiserum diluted 1:1000 were labeled by the avidin-biotin peroxidase technique, using biotinylated Protein A as the bridging reactant. The human pituitary adenoma biopsies were obtained from three groups of patients: acromegalics with elevated circulating growth hormone (GH), amenohorrea/galactorhorrea patients with elevated serum prolactin (PRL) levels, and patients with clinically non-secreting adenomas. Sections adjacent to motilin-reacted ones were immunostained with antisera to human GH and ovine PRL. Motilin reactivity was always present in somatotropic regions of the guinea-pig and human gland, and rims of normal adenohypophysis next to the adenomas. In many instances it was co-localized in individual somatotrophs traced in adjacent sections stained for GH. No motilin reactivity was detected in the 9 non-secreting or 6 prolactin secreting tumors. Motilin staining however, was demonstrated in 7 of the 14 GH secreting adenomas. It was not affected by pre-absorption of the final dilution of antibody with GH, and was completely or almost completely blocked by 100 ug of synthetic porcine motilin. These experiments demonstrate that a motilin like peptide is characterestically present in the cytoplasm of both normal and neoplastic somatotrophs. The coincidence of GH and motilin reactivity in single cells, however, was not constant, suggesting that at any given moment the content of the two peptides in a somatotroph may not be parallel. Motilin reactivity on the other hand, was generally less intense than GH's and the observed discrepancies may be explained in part, by relative insensitivity of the motilin antiserum.

(Supported by NIH Grants NS18324 and NS18335, and a Parkinson's Disease Foundation Grant to Columbia University).

294.7 ORIGIN AND DISTRIBUTION OF IMMUNOREACTIVE CORTICOTROPIN RELEASING FACTOR (irCRF) IN THE RAT SPINAL CORD. M.A. Hynes*, I. Merchenthaler* and P. Petrusz* (SPON: R.A. King). Dept. of Anatomy, University of North Carolina, Chapel Hill, North Carolina 27514.

Using immunocytochemistry we have reported the presence of irCRF fibers in the lateral white matter of the rat spinal cord. Following a single complete transection at low cervical levels the extensive accumulation of irCRF distal to the cut indicated that most of these fibers were ascending. A few irCRF cell bodies were seen in laminae VI, VII and X in long-term hypophysectomized rats

To discern whether this ascending fiber system represents a single projection or is a network of propriospinal neurons, two complete transections at a distance of a few segments were made A dense accumulation of irCRF was observed distal to each cut in the lateral column. The fibers occupied a well defined area of the dorsal lateral column, tapering off at deeper levels and disappearing at a level corresponding to lamina V-VI of the gray matter. This pattern of accumulation was observed in both the distal and the isolated segments The accumulation of irCRF in the rostral aspect of the isolated segment indicated that this population of CRF fibers originated from perikarya within the isolated segment or from the periphery. However, irCRF cell bodies or fibers were not observed in dorsal root ganglia.

After local injection of colchicine into the spinal cord (250 µg), irCRF cells were seen distributed along the intermediolateral column, in lamina X, and in the marginal zone (MZ) of the dorsal horn. The cells of the MZ were of the bipolar type oriented in the rostal-caudal direction. These cells are especially evident in parasagittal sections. There is also a dense network of irCRF fibers in the MZ.

Hypothalamic CRF has been recognized as a mediator of stress-related responses. The presence of this peptide in the MZ of the spinal cord makes it interesting to compare its distribution in this region to that of peptides purported to influence transmission of pain signals at the spinal level Fibers with irCRF, although dense adjacent to the dorsal horn in the lateral column and present in the dorsal column, are much more discretely localized than enkephalin; enkephalin fibers traverse the dorsal horn at many orientations and perikarya are scattered in many areas. Finally, in contrast to CRF, enkephalin shows a large accumulation both proximally and distally to the cut in a spinally transected animal.

Supported in part by the Neurobiology Program, University of North Carolina, Chapel Hill, and by USPHS Grant No. NS14904.

294.8 AUTORADIOGRAPHIC LOCALIZATION OF TRITIATED ANTIBODY IN DORSAL HORN. E.J. Glazer, J. Ramachandran* and A.I. Basbaum. Department of Anatomy and Hormone Research Laboratory, University of California, San Francisco, CA 94143.

To sort out the complex synaptic circuitry in the spinal dorsal horn, we have developed a histochemical method using autoradiographic localization of tritiated goat antirabbit IgG (^{3}H-GAR) second antibody. This study describes the methodology and compares it with PAP immunocytochemistry for the demonstration of substance P (SP)

^{3}H-GAR was prepared by incubating affinity purified GAR (E Y. Labs.) with ^{3}H-propionyl succinimidate (New England Nuclear). Following incubation, the ^{3}H-GAR was separated from unlabelled GAR on a Sephadex G-50 column.

For anatomical studies, paraffin sections of cat or rat spinal cord were blocked in 3% goat serum and then incubated in dilutions of SP antiserum (Immunonuclear Corp.), ranging from 1/500 - 1/5000, for 48 hours at 4^{0}C. The sections were then blocked in 3% goat serum and incubated for one hour at room temperature in ^{3}H-GAR (100,000 cpm/0.2ml). Following extensive washing, the sections were mounted on slides, coated with Kodak NTB-2 emulsion and exposed in the dark. Optimum exposure times ranged from 5 days to two weeks. The resulting distribution of SP on the autoradiograms was identical to the distribution of SP observed with PAP immunocytochemistry; silver grains were concentrated in laminae I and outer II with smaller patches of immunoreactivity present in lamina V. The optimum dilution of SP antibody for the autoradiographic demonstration of SP was 5 times greater than that needed for the PAP reaction indicating a greater sensitivity of the ^{3}H-GAR method. Absorption controls demonstrated the specificity of the autoradiographic label for SP; 100ug of synthetic SP added to the primary antiserum abolished the dense accumulation of silver grains in the superficial dorsal horn.

This autoradiographic procedure can be used with peroxidase histochemistry to double label immunoreactive elements at the ultrastructural level. It is also quantifiable at the light microscopic level, so that experimentally induced changes in the distribution of peptides in localized areas of the CNS can be measured.

Supported by NSF BNS8104482, DA01949 and NS14627.

294.9 LOCALIZATION OF CRF-LIKE IMMUNOREACTIVITY IN THE CAUDAL NEUROSECRETORY SYSTEM OF SEVERAL SPECIES OF FISH D. Onstott* and R. Elde, Departments of Ecology and Behavioral Biology and Anatomy, University of Minnesota, Minneapolis, Minnesota 55455

Two peptides exhibiting various biological effects have been isolated from the caudal neurosecretory system of fish, and others may be present. One of these peptides, urotensin I (UI), produces a pronounced lowering of blood pressure in mammals and birds, but little is known about its specific role in the physiology of fish. In addition, it has not been possible to localize UI to neurosecretory cells within this system nor to characterize anatomically or histochemically the specific regulatory mechanisms involved in its secretion. Recently the amino acid sequence of UI has been determined (Lederis et al., Science 218:162, 1982) and has been shown to exhibit a strong homology with ovine corticotropin releasing factor (CRF). Using an antiserum to ovine CRF, we have demonstrated the presence of strong CRF-like immunoreactivity in the caudal spinal cord and urophysis of the channel catfish (Ictalurus punctatus).

Fish were perfused with 4% paraformaldehyde in 6.5 pH phosphate buffer then 4% paraformaldehyde in 9.2 pH borate buffer. Cryostat sections (10 μ) of the caudal spinal cord and urophysis were processed using the indirect immunofluorescence method with primary antiserum directed against synthetic ovine CRF.

Beginning with sections taken 2-3 segments rostral to the urophysis, large dorsally located immunoreactive cell bodies and ventrally located fibers, some with decussations, can be seen. Often large processes are visible as they leave the cell bodies. More caudally, the number of immunoreactive cell bodies increases until they fill most of the dorsal two-thirds of the spinal cord. Just anterior to the urophysis, fibers form a compact, highly fluorescent tract ventrally, while the number of fluorescent cell bodies is reduced. The urophysis itself is filled with a highly fluorescent network of fibers, while the spinal cord dorsal to it contains only a few immunoreactive cell bodies. Adjacent sections pretreated with an excess of CRF showed no staining. Similar results were obtained with the cyprinid species Notemigonus chrysoleucas and Catastomus commersoni.

The caudal neurosecretory system receives descending monoaminergic and possibly cholinergic input, and some projections have recently been traced from the midbrain and medulla (O'Brien and Kriebel, Cell Tissue Res. 227:153, 1982). The ability to localize a particular peptide to the neurosecretory cells within this system will make it possible to characterize the neuronal circuitry and chemical substances involved in the control of its secretion.

Supported by ImmunoNuclear Corp. and DA 02148.

294.10 SUPRACHIASMATIC EFFERENT PROJECTIONS TO THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS: ULTRASTRUCTURAL IMMUNOCYTOCHEMISTRY AND SYNAPTOLOGY. S. Tallaksen* and D.T. Piekut. (SPON: C. Ferrario). The Neuroendocrine Unit, Univ. of Rochester, Rochester, NY 14642.

The neuroanatomical localization of suprachiasmatic vasopressinergic fiber projections to the paraventricular nucleus (PVN) of hypothalamus was examined by combined anterograde degeneration and immunocytochemistry. Electrolytic lesions were made with a direct anodal current of 0.3-0.5mA for 10-20 seconds into the suprachiasmatic nucleus (SCN) using an approach from the contralateral side. Following a survival period of 1-3 days, animals were anesthetized and brains fixed by cardiac perfusion. Immunocytochemical localization of vasopressin, oxytocin and neurophysin, using the avidin-biotin complex (ABC) method, was performed on thick 50μ Vibratome sections from both experimental and control animals. Immunostained thick sections were then blocked and processed for electron microscopy.

We have previously reported that discrete, unilateral lesions of 80-100% of the SCN results in a marked ipsilateral reduction or disappearance of fine-caliber vasopressin-immunoreactive fibers in specific areas of rat forebrain. The present study demonstrates a marked ipsilateral reduction of immunopositive fibers in functionally distinct subnuclei of the parvocellular region of the PVN complex, specifically the periventricular, dorsal and medial subdivisions. At the electron microscopic level, electron-dense degeneration is characterized by a darkening of the axon terminal, increased packing of synaptic vesicles and decreased definition of organelles.

Preliminary data suggest a structural relationship between PVN neurons and SCN vasopressinergic projections. Immunoreactive neurophysin, oxytocin and vasopressin cells are identified predominantly in the magnocellular region of the PVN complex. Ultrastructure characteristics of neurophysin and oxytocin immunostained neurons are similar to that described for vasopressin-containing cells. Immunoreactive material is observed within the perikarya and neuronal processes. Symmetrical and asymmetrical synaptic contacts are seen; axo-somatic, axo-dendritic and putative axo-axonal contacts are identifed. Presynaptic profiles contain mitochondria and clear vesicles or a mixture of clear and dense cored vesicles. Our identification of synaptic contacts on neurophysin and oxytocin immunoreactive cells provides an anatomical substrate for afferent input to magnocellular neurons of the PVN.

294.11 THE PARAVENTRICULAR NUCLEUS: AFFERENT FIBER SYNAPTOLOGY. D.T. Piekut and S. Casey*. The Neuroendocrine Unit, Univ. of Rochester, Rochester, NY 14642.

The anatomical and functional complexity of the paraventricular nucleus (PVN) of hypothalamus is now recognized. A vast number of neuropeptides have been identified within the PVN complex which suggests that this nucleus is emerging as a central site for the integration and coordination of some neuroendocrine and autonomic activities. This study addresses potential interactions and communications between cells of the PVN and the role of afferent fiber projections in influencing PV neuronal (e.g. vasopressin) functions.

Anatomical details have been elucidated using Vibratome tissue sections of rat brain, the basic and double antigen ABC method of immunocytochemical staining at the light microscopic level, and the immunocytochemical and pre-embedding staining procedures at the electron microscopic level. The localization of immunoreactive opiocortin fibers and CRF, enkephalin and somatostatin perikarya within specific functionally distinct subnuclei of the parvocellular division of the PVN is identified. A structural relationship between parvocellular and magnocellular cells has been suggested. Vasopressin containing neurons are identified by light microscopy predominantly in the postero-dorsolateral portion of the posterior magnocellular division of the PVN. By EM, the immunoreaction product is seen within the cell body and neuronal processes. In the perikaryon, immunoreactive material is associated primarily with neurosecretory granules. Immunostained cell bodies and neuronal processes are frequently wrapped in glial lamellae. Synaptic contacts are observed with immunostained vasopressinergic neurons; axo-somatic, axo-dendritic and putative axo-axonal synapses are identified. The unlabeled pre-synaptic element contains mitochondria and clear vesicles or a mixture of clear and dense cored vesicles; symmetrical and asymmetrical synapses are noted. The amount and proportion of microtubules and neurosecretory granules observed within neuronal processes identified as axons is variable; immunoreaction product is seen in association with both types of organelles. This study provides an anatomical substrate for functional interactions to occur between magnocellular neurons of PVN and local or afferent fibers which project to the PVN complex.

294.12 NEW HYPOTHALAMIC SITES FOR IMMUNOREACTIVE SOMATOSTATIN PERIKARYA. C. Bennett-Clarke* (SPON: R.K. Pettegrew). The Neuroendocrine Unit, Univ. of Roch., Rochester, NY 14642.

The immunocytochemical distribution of SRIF (growth hormone-inhibitory release hormone) was examined in colchicine treated adult male rats. Colchicine (100μg) was infused stereotaxically into the lateral ventricle in a saline vehicle. Following a 48 hr. survival time animals were intracardially perfused with Bouin's fixative and brains were serially sectioned on a Vibratome. Sections were immunocytochemically stained using the unlabeled antibody enzyme method with SRIF antiserum, Charlie "8", diluted at 1:1000.

The number and the intensity of staining of SRIF positive, perikarya was increased following colchicine administration throughout the entire forebrain of the rat. Within the preoptic and hypothalamic regions the largest accumulation of SRIF perikarya was in the periventricular stratum. In colchicine treated animals these cells extended, rostral to caudal, from the nucleus of the diagonal band of Broca through the posterior portions of the arcuate nucleus. SRIF immunoreactive cells were demonstrable within the medial and lateral preoptic nuclei, the suprachiasmatic nuclei, all portions of the periventricular nucleus, the parvocellular portions of the paraventricular nucleus, the anterior hypothalamic nucleus, the dorso- and ventromedial nuclei, the arcuate nucleus and the ventral premamillary nucleus. Additionally SRIF perikarya were noted in the retrochiasmatic, zona incerta and perifornical regions, in the cell space area surrounding the ventromedial nucleus, within the lateral hypothalamic area and extending from there ventrally along the base of the brain. These data represent many "new" sites for SRIF neurons within the hypothalamus and offer new possibilities for the intrahypothalamic connectivity of the SRIF system.

294.13 IMMUNOCYTOCHEMICAL EVIDENCE THAT GRF 1-44-NH_2 IS LOCALIZED IN THE TUBEROINFUNDIBULAR SYSTEM OF RHESUS MONKEY HYPOTHALAMUS. R.M. Lechan, H.D. Lin*, N.C. Ling, and S. Reichlin. Endocrine Division Tufts-New England Medical Center, Boston, MA 02111, and Neuroendocrinology Laboratory, Salk Institute, La Jolla, CA 92138.

Recent reports by Guillemin et al (Science 218, 585, 1982) and Rivier et al (Nature 300, 276, 1982) have described the sequence of a family of peptides with potent growth hormone releasing activity, isolated from human pancreatic islet cell tumors. Using an antiserum directed against the carboxyl terminus of synthetic growth hormone-releasing factor (GRF_{1-44}-NH_2), we describe the distribution of GRF in the hypothalamus of the rhesus monkey.

Synthetic GRF_{1-44}-NH_2 was conjugated to methylated BSA and used to immunize NZW rabbits. Antiserum from one rabbit (#850) reacted with GRF_{1-44}-NH_2 giving a sensitivity of 156 pg/tube at a titre of 1:4000 in the RIA and showed < 0.1% cross-reactivity with synthetic GRF_{1-40}-OH. Immunocytochemical studies were performed on 50 µm coronal sections of rhesus monkey hypothalamus, fixed en bloc for 2 hrs in 10% acrolein in 0.1M Sorensen's phosphate buffer, pH 7.2. To improve visualization of neuronal perikarya, axonal transport was inhibited in 2 animals by the intraventricular administration of colchicine 24 hrs prior to killing. Antiserum to synthetic GRF_{1-44} was used at a titre of 1:800, diluted in Tris buffered saline-HCl, pH 7.6, containing 0.3% Triton X-100. The reaction product was developed with DAB, following the indirect peroxidase-antiperoxidase technique.

Intense reaction product was present throughout the rostral-caudal extent of the median eminence and stalk beginning as two separate fiber bundles at the lateral margins of the median eminence in rostral portions and joining in the midline in caudal portions. Terminals were largely concentrated in the external zone of the median eminence, juxtaposed to the portal capillaries. Lesser amounts of immunoreactivity was also present in the external zone in mid-regions of the median eminence, in the arcuate nucleus and in rare beaded fibers in the periventricular nucleus but no other region of the hypothalamus. In colchicine treated animals, numerous immunoreactive perikarya were visualized in the arcuate nucleus, particularly in posterior regions and in portions of the ventromedial nucleus. All immunocytochemical staining was completely abolished by preincubation of the antiserum with 10^{-6}M synthetic GRF_{1-44}-NH_2 but not by 10^{-5}M of the free acid forms of GRF 1-44, 1-40, 1-37, 1-34, 1-31, 1-28 or 1-24.

These findings demonstrate that in the hypothalamus, GRF is distributed extensively in cells and fibers corresponding to the tuberoinfundibular system. In contrast to other hypophysiotropic factors found in the hypothalamus, GRF may function solely as a pituitary regulating factor. In addition, the high specificity of the antiserum used to demonstrate GRF in this study is strong evidence that GRF_{1-44}-NH_2 is present in the primate hypothalamus.

294.14 ANALYSIS OF EFFERENT PROJECTIONS FROM THE ARCUATE OPIOCORTIN SYSTEM IN RAT BRAIN. G.J. Michael* and S.A. Joseph. (SPON: J. Way). The Neuroendocrine Unit, Univ. of Rochester, Roch., NY 14642.

Anatomical studies have localized ACTH and other opiocortin peptides in rat brain. The perikarya of this system have been located only in the mediobasal hypothalamus. The fibers which project from these neurons have an extensive distribution in hypothalamic, thalamic and limbic areas as well as in the brainstem periaqueductal gray (PAG) and other brainstem nuclei. This study utilizes knife cuts and electrolytic lesions to identify the various efferent pathways of this system into the brainstem.

Observations of normal rat brain sections stained immunocytochemically for ACTH indicates several bundles of fibers originating from perikarya in the arcuate opiocortin bed nucleus. Most noticeable are medial fiber bundles in the dorsal longitudinal fasciculus and periventricular tract which appeared to be responsible for the dense innervation of forebrain and thalamic areas and PAG. Another group of fibers is found more lateral and represents an additional projection to the PAG. This pathway is in the medial forebrain bundle, passes dorsal to the substantia nigra, around the geniculate bodies and finally between the geniculate region and PAG. Lesions were made to interrupt these various fiber bundles. Five to 10 days following surgery brains were fixed in Bouin's, sectioned at 50µm with a Vibratome and stained immunocytochemically for ACTH.

The results of these lesion studies indicate that there are four major ACTH efferent projections from the mediobasal hypothalamus. (1) Fibers coursing rostrally innervate forebrain nuclei and some extend medially and dorsally around the thalamus in the dorsal longitudinal fasciculus. These latter fibers innervate thalamic nuclei. (2) Fibers which are observed caudally in the periventricular tract bifurcate. One component extends in a rostral direction around the caudal extent of the thalamus and innervate thalamic nuclei. The other components continue caudally to PAG and brainstem. (3) In the preoptic and anterior hypothalamic regions a group of fibers project laterally to medial amygdaloid nuclei. (4) Fibers in the median forebrain bundle project in a dorsal lateral direction over the substantia nigra to areas around the geniculate bodies and finally project medially to the PAG.

294.15 LHRH IN NERVUS TERMINALIS, OLFACTORY BULBS AND VOMERONASAL ORGAN OF THE RAT, J. W. Witkin and A-J. Silverman. Dept. Anatomy & Cell Biology, Columbia U., Coll. P.& S., New York, NY, 10032.

Luteinizing hormone-releasing hormone (LHRH) systems of rat olfactory bulbs and nasal areas were studied in neonatal and adult rats. Animals were perfused with Zamboni's fixative and nasal regions with olfactory bulbs in situ were postfixed and decalcified. LHRH was demonstrated immunohistochemically in either frozen or unembedded vibratome sections.

LHRH-immunoreactive elements were found along the course of the nervus terminalis (NT), within the main and accessory olfactory bulbs, along the lateral olfactory tract and associated with the olfactory epithelium of the vomeronasal organ. The distribution of LHRH neurons was sparse. There were LHRH neurons associated with central portions of the NT in its course along the anterior cerebral artery in the olfactory tubercle in adult and neonatal rats. Occasional immunoreactive neurons were found in the pia or subarachnoid space along the AOB in adult and neontal animals and along the MOB in neonatal animals. There were no ganglia of LHRH positive neurons along the NT. There were a few LHRH neurons along the course of the lateral olfactory tract, both lateral to it and medially, along the accessory olfactory tract in its route to the medial amygdala. LHRH neurons were found in the accessory olfactory bulb (AOB) and medial portion of the anterior olfactory nucleus, but not in the main olfactory bulb (MOB). LHRH-immunoreactive fibers were found in all laminae of the AOB but mainly in the external plexiform layer of the MOB. LHRH fibers accompanying the NT were seen to penetrate the surface of the AOB and the MOB.

The source of the LHRH innervation of the AOB is probably from intrinsic neurons, the NT, and neurons along the course of the accessory olfactory tract. LHRH fibers in the MOB probably have their source in the anterior hippocampal rudiment, the anterior olfactory nucleus, the NT, and neurons along the lateral olfactory tract. Other possible sources of LHRH innervation of the MOB based on earlier projection studies (de Olmos, J. et al., J. Comp. Neurol. 181:213, 1978 and Davis, B.J. et al., Br. Res. Bull. 3:59, 1978) are the septal area and diagonal band of Broca. Similarly, there may be LHRH innervation of the AOB by the bed nucleus of the stria terminalis. Although LHRH cells and fibers have been observed in all of these loci, (Witkin, J.W. et al., Neuroend. 35:429, 1982) it was not possible to trace LHRH fibers completely from these areas to the olfactory bulbs. The source of LHRH innervation of the vomeronasal organ could not be traced in its entirety, but may be the NT as LHRH fibers were seen accompanying the anteriorly projecting course of the NT at the surface of the AOB and were seen within the nasal septum.

294.16 POSSIBLE DIFFERENCES IN THE DYNAMICS OF MOLECULAR PROCESSING OF LHRH IN RATS VS. OTHER MAMMALS SUGGESTED BY IMMUNOCYTOCHEMICAL STUDIES. J.C. King and E.L.P. Anthony*. Dept. of Anatomy and Cellular Biology, Tufts University Schools of Medicine, Boston, MA 02111.

Biochemical studies of the molecular processing of the hypothalamic peptide Luteinizing Hormone Releasing Hormone (LHRH) have proven to be extremely difficult; this is possibly due to the small percentage of neurons that contain the hormone together with their dispersed loci in the forebrain. Studies employing immunocytochemical methods for the localization of various moieties of LHRH in situ may contribute to a better understanding of the biosynthesis and maturation of this hormone. We have examined the moieties of immunoreactive LHRH present within neuronal perikarya and fibers in several species: humans, rhesus and cynomolgos monkeys, bats, ferrets, and rats. One primary antiserum used in these studies (Arimura's R422) demonstrates a distinctive binding requirement for the free amino- and carboxy- peptide terminals of LHRH; these terminals would be available for binding in the mature decapeptide. The second antiserum used (Arimura's R419) requires interior amino acid sequences, which would be available both in the mature decapeptide and in a possible precursor molecule which may be extended at both terminals. LHRH within perikarya of rats was not immunoreactive with the antiserum requiring the free decapeptide terminals (R422), but was reactive with the antiserum which would react with a larger peptide (R419). In humans, monkeys, bats, and ferrets, however, the presence of decapeptide within perikarya was strongly suggested by immunoreactivity of the LHRH moieties with R422. Free decapeptide was apparently present in all species in terminal regions of the processes at the neurovascular contact zone of the median eminence. These results suggest that the rat may not convert LHRH to the physiologically active hormone within the perikaryon of the neuron, but rather during transport along the processes or in neuronal terminals. In several other species however, including humans, this conversion seems to occur within the perikaryon and the hormone can be stored within cytoplasmic granules. This immunocytochemical evidence suggests differential dynamics of the molecular processing of LHRH within neurons in rats vs. other mammals, including primates. This work was supported by grants HD00352 from NIH and PCM 8103243 from NSF to JCK.

294.17 IMMUNOHISTOCHEMICAL AND AUTORADIOGRAPHIC ANALYSIS OF THE MEDIOBASAL HYPOTHALAMUS OF RATS TREATED NEONATALLY WITH MONOSODIUM GLUTAMATE. L.Jennes*,W.E.Stumpf,G.Bissette and C.B.Nemeroff,Dept.Anat.and Biol.Sc.Res.Ctr.,Univ.of North Carolina,Chapel Hill, NC 27514.

The effects of neonatally administered monosodium glutamate (MSG) on the distribution of immunoreactive tyrosine hydroxylase, neurotensin, glutamic acid decarboxylase (GAD) and gonadotropin-releasing hormone (GnRH), as well as on nuclear uptake of ^{3}H-estradiol have been studied by immunohistochemistry and autoradiography. Neonatal male and female rats received 4 mg/g bw MSG intraperitoneally at days 2,4,6,8,and 10.At day 60, the aminals were colchicinized (intracisternal injection of 50 ug/100 g bw colchicine), 48 hrs before ether anesthesia and sacrifice by intracardiac perfusion with 4% paraformaldehyde. Immunohistochemistry was performed on vibratome as well as on paraffin sections using Mason's single bridge technique. Estradiol was localized with thaw mount-autoradiography after intravenous injection of ^{3}H-estradiol, 0.2ug/100g bw. After MSG treatment, a substantial reduction in the number of neuronal cell bodies in the arcuate nucleus of the hypothalamus could be noted. Immunohistochemistry for tyrosine hydroxylase reveals a dramatic decrease in dopaminergic neurons in the ventral and ventrolateral portion of the nucleus arcuatus, while some positively stained perikarya remain visible in the dorsomedial aspect of the nucleus close to the ventricular surface. Dopaminergic fibers, although decreased, remain numerous in the external layer of the median eminence. Neurotensin immunoreactive structures show very similar features, i.e. the number of perikarya is largely reduced in the arcuate nucleus, though few cell bodies remain visible ventrolateral to it . In the median eminence, neurotensin-immunoreactivity is reduced. Also, GAD-immunoreactive nerve fibers are substantially decreased in number in the original area of the arcuate nucleus throughout its extent, as well as in the median eminence, which shows only very few GAD-positive nerve fibers left after the treatment. In contrast, the GnRH-containing nerve fiber system is not noticeably affected by the treatment. Especially in the median eminence, the distribution pattern of GnRH fibers appears intact. Autoradiography to visualize nuclear uptake of ^{3}H-estradiol by neurons of the arcuate nucleus reveals an almost complete loss of estrogen target cells in this area of treated animal, when compared with control animals. The results suggest that MSG toxicity affects different neurotransmitter and neuropeptide-producing neurons, although differentially. The resulting disturbance of the neuroendocrine regulation of LH and FSH secretion does not seem to be due to a direct effect of MSG on the GnRH-system, and is probably caused by the elimination of actions of other regulatory factors, including, the tuberoinfundibular systems of dopamine, GABA,neurotensin,and estradiol target neurons. Supp. by PHS grants NSO9914, NS17614, NIMH MH-34121 and NICHHD HD-03110.

294.18 IMMUNOCYTOCHEMICAL MAPPING OF IMMUNOREACTIVE PROLACTIN IN FEMALE RAT BRAIN. B. D. Shivers*, R. E. Harlan* and D. W. Pfaff (SPON: P. Femano). The Rockefeller University, New York, NY 10021.

Cells and fibers containing immunoreactive prolactin (ir-PRL) were localized in female rat brain and spinal cord. This extends the description of neural circuitry which may mediate the facilitatory effects of estrogen on the lordosis reflex (Harlan et al., Science 219:1451, 1983).

Female rats were perfused with buffered picric acid-paraformaldehyde or neutral-buffered paraformaldehyde. Sections were incubated in rat prolactin antiserum (National Hormone and Pituitary Program: A. Parlow; 1:500 to 1:2000 in 0.2% Triton-PBS). The reaction product was localized using the peroxidase-antiperoxidase method or the biotin-avidin system. Controls: When the primary antiserum was substituted with normal serum or when preabsorbed with 10 μM prolactin, no specific staining was seen. Also, specific staining was not eliminated by preabsorption of the primary antiserum with ACTH 1-24, β-endorphin, follicle-stimulating hormone, growth hormone, β-lipotropic hormone, luteinizing hormone, or met-enkephalin. Finally, ir-PRL was seen in cell bodies and fibers in hypophysectomized female rats.

Cell bodies containing ir-PRL were found exclusively in the mediobasal hypothalamus (MBH): in the arcuate nuclei and in a band extending lateral and ventral to the ventromedial nuclei and the mamillary recess. Ir-PRL cell bodies, estimated at 2000-3000 per colchicine-treated brain, were found in all coronal sections from the rostral tip of the arcuate nuclei to the most caudal extent of the mamillary recess.

Fibers containing ir-PRL and arising from MBH cell bodies extended rostrally into the anterior hypothalamus, preoptic area, bed nucleus of the stria terminalis, septum, diagonal bands of Broca and nucleus accumbens. Laterally-directed fibers crossed the supraoptic commissures and extended into the amygdala. Other fibers travelled to the midbrain central gray taking pathways either: a) around the lateral-dorsal thalamus, b) through the peripeduncular region, or c) near midline. Fibers also continued caudally to enter the parabrachial region, or travelled dispersely in the reticular formation throughout the lower brainstem. Fibers containing ir-PRL were found at all levels of the spinal cord, especially in the ventral horn region.

294.19 SUPRAMAMMILLARY AFFERENTS TO GUINEA PIG HIPPOCAMPUS CONTAIN SUBSTANCE P-LIKE (SP) IMMUNOREACTIVITY. C. Gall and L. Selawski*, Department of Anatomy, University of California, Irvine.

A complex pattern of substance P-like (SP) immunoreactivity is observed in the guinea pig hippocampus. Immunoreactive axons are found within the hilus, around the pyramidal cell bodies of regio inferior, and most densely in the supragranular and superficial granule cell fields of the dentate gyrus. Colchicine treatment reveals numerous SP positive perikarya within the hilus and fewer, more faintly immunoreactive, perikarya in the cell body and molecular lavers of regio inferior. In the present study the source of SP immunoreactive axons in guinea pig hippocampus was studied using transection, fluorescent dye transport, and immunocytochemical techniques.

In three guinea pigs (Hartly strain) the rostral hippocampus and fimbria were transected near the septum three weeks before sacrifice. This treatment severely reduced the density of immunoreactive axons in the ipsilateral (but not contralateral) hippocampus and virtually eliminated the supragranular immunoreactive axonal group. This transection severs the diencephalic, brainstem, septal and hippocampal commissural afferents of the ipsilateral hippocampus.

In order to determine which of these areas gives rise to hippocampal SP axons, the fluorescent dye fast blue was injected into four loci (in the vicinity of stratum granulosum) within the rostral half of one hippocampus in seven guinea pigs. Forty-eight hours later, 90 ugm colchicine was injected into the lateral ventricle. The animals were sacrificed 15 to 24 hours later. Fast blue labeled neurons were found in the septum, supramammillary region, contralateral hippocampus, ipsilateral entorhinal cortex, raphe, nucleus reuniens thalami, and reticular formation. Immunocytochemical processing of the same tissue sections demonstrated numerous fast blue labeled cells in the supramammillary region also contained SP immunoreactivity. Double labeled cells were present bilaterally with greater frequency ipsilateral to the injected hippocampus. SP/fast blue labeled cells were absent in all other regions giving rise to hippocampal afferents.

These data indicate that most axonal substance P-like immunoreactivity in the hippocampal formation of the guinea pig is contained within axons from the supramammillary region. It seems likely that this is also true in other animals (cat, squirrel, monkey) sharing a similar pattern of hippocampal substance P. In light of these findings it will be of interest to determine the pharmacological properties of the supramammillary afferents of hippocampus in these animals.

Supported by NSF grant BNS82-00319 and an Alfred P. Sloan Fellowship to C.G.

294.PO DISTRIBUTION OF ACTH AND ALPHA MSH-SPECIFIC PERIKARYA IN THE EQUINE DIENCEPHALON. P.A. Melrose* and K.M. Knigge. The Neuroendocrine Unit, Univ. of Rochester, Rochester, NY 14642.

The distibution of αMSH and ACTH$_{1-39}$ immunoreactive (-ir) neurons was mapped in the diencephalon of 3 horses and 2 ponies. Brains were fixed in buffered 4% paraformaldehyde containing 0.1% picric acid. Serial 50μm sections were cut on a freezing sliding microtome and alternate sections were stained with antisera specific for ACTH and αMSH using unlabeled antibody techniques. Two groups of ir-cells were distinguishable based on distribution and cross immunoreactivity. Perikarya of the first system stained with antisera to αMSH and to ACTH (1-39), and were localized in the periventricular areas of the arcuate nucleus. Based on the topography of these perikarya and their fiber distribution, this network appears to be equivalent to the arcuate opiocortin system described in other species. The second pool of cells stained only with antisera generated against αMSH. Anteriorly these perikarya have a specific distribution which extends from perifornical areas of the dorsomedial nucleus and the lateral hypothalamus into the zona incerta. Posteriorly this system divides into a perifornical group and a posterior hypothalamic group. The perifornical group of cells extends to the level of the premammillary nuclei whereas the dorsal group continues into the nucleus supramamillaris.

295.1 STUDIES ON THE STRUCTURE OF THE AXONEME OF OLFACTORY CILIA IN FROGS AND RATS USING TANNIC ACID-SUPPLEMENTED FIXATION AND PHOTOGRAPHIC ROTATION. M. S. Lidow, B. Ph. M. Menco, R. C. Gesteland and A. I. Farbman. Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201.

Previous studies suggested that some vertebrate species have olfactory cilia containing a full complement axoneme in their proximal segments, i.e., a (9x2) + 2 microtubular configuration and doublet-attached arms and spokes. In other species, particularly in mammals, arms and spokes are lacking. Moreover, as reported in a previous study from this laboratory, motile and immotile cilia coexist in the frog olfactory epithelium. Since doublet-attached arms are associated with motility, it was of interest to examine the fine structure of frog olfactory cilia to determine whether motile and immotile cilia exhibited differences. In addition we examined rat olfactory cilia and respiratory cilia from both species for comparative purposes. The electron microscopic procedures included two techniques to enhance visualization of axonemal features: tannic acid-supplemented aldehyde fixation and photographic rotation. The ninefold radial symmetry of cilia was photographically enhanced by rotating the print 5 complete revolutions so that each print had a total of 45 very brief exposures. The results show clearly that the axonemes in the proximal segments of frog olfactory cilia resemble those of respiratory cilia in all respects; with these methods distinct types of olfactory cilia corresponding to motile and immotile ones could not be discerned. In contrast to the frog, rat olfactory ciliary axonemes were found to lack arms and spokes. Distal segments of all cilia examined in both species contained only microtubules; these distal segments were much longer in olfactory than in respiratory cilia. Tannic acid-supplemented fixation brought out fairly consistent differences between membranes of olfactory and respiratory cilia in both species, i.e., outer leaflets of the bilayer were thicker in olfactory cilia whereas inner leaflets were thicker in respiratory cilia. These findings corroborate previous freeze-fracture studies with respect to membrane differences. We show that all olfactory cilia do not have a full complement axoneme and that axonemes probably serve as a cytoskeletal support for the receptor cell membrane to increase the area of the receptive surface.

Supported by NIH grants #NS-18490, NS-07223, NS-14663, and NSF grant BNS-8117075.

295.2 A MONOCLONAL ANTIBODY DETECTS A CELL SURFACE GLYCOPROTEIN FAMILY FOUND ON OLFACTORY RECEPTOR NEURONS W.K. Allen* and Richard Akeson. Div. of Cell Biology, Children's Hosp. Res. Fndn., Univ. of Cincinnati, Cincinnati, OH 45229.

The study of nervous system development can be facilitated by the use of neuron subclass specific monoclonal antibodies (Mab's). We have produced a Mab which recognizes a cell surface glycoprotein family specific to sensory receptor neurons of the olfactory epithelium. The 2B8 IgG_3, κ secreting hybridoma cell line was produced by the fusion of X63-Ag8.653 myeloma cells and spleen cells from a Balb/c mouse immunized with the neural crest derived cell line, PC12 (a rat pheochromocytoma). Mab binding to particulate protein from adult rat dorsal root ganglion and some non-olfactory areas of the CNS can be readily detected by radioimmune assays (RIA). However the reactivity with 2B8 in immunofluorescence analyses of cryostat sections of dorsal root ganglion, cerebellum and hippocampus was near or at background (nonimmune IgG) levels. Olfactory bulb and olfactory epithelium demonstrate binding 5-6 fold greater than that of any other area of the nervous system by RIA. Indirect immunofluorescence on cryostat sections of olfactory bulb shows intense binding in the olfactory nerve layer (consisting primarily of receptor cell axons), the glomerular layer (area of receptor cell synapse formation within the olfactory bulb) and the glomerular layer of the accessory olfactory bulb. In the olfactory neuroepithelium there is a continuous process of receptor cell renewal. This involves the differentiation of proliferative basal cells, neurite outgrowth, synapse formation in the glomerular layer of the olfactory bulb, and finally senescence. Indirect immunofluorescence localization of the 2B8 antigen in the olfactory epithelium demonstates its presence on a subset of receptor cells. 2B8 Mab also binds to a subset of round cells in the basal cell layer. Portions of the epithelium consist of a single layer of characteristic short tapered cells all of which immunofluoresce strongly. The vomeronasal organ shows significant binding except in regions of respiratory epithelium. Supporting cells of the olfactory epithelium do not appear to express the 2B8 antigen. Analysis of 2B8 binding to SDS polyacrylamide gels of olfactory bulb detergent soluble membrane proteins indicates a family of 3 to 4 proteins with M_r's between 70K-150K. Polypeptides of similar M_r's were immunoprecipitated from ^{3}H-glucosamine biosynthetically labelled PC12 membrane proteins indicating the glycoprotein nature of the antigens. The immunofluorescence data with olfactory epithelium and bulb suggest this family of glycoproteins may be a unique cell surface marker for sensory receptor cells during their development.

295.3 EXPERIMENTALLY INDUCED EXTRAEPITHELIAL MIGRATION OF OLFACTORY NEURONS IN NEONATAL AND ADULT RODENTS. G.A. Monti Graziadei* and P.P.C. Graziadei. (SPON: J. Elam). Florida State University, Tallahassee Fla 32306.

Migration of neuronal elements from the olfactory neuroepithelium takes place during ontogenesis. This phenomenon, never reported in adult animals, has been now observed to occur as a result of stressful experimental conditions.

Our observations have been conducted on neonatal and adult albino mice (NIH-CD-1) and rats (Sprague-Dawley) partially or totally unilaterally bulbectomized. After fixation by perfusion in Bouin's liquid and decalcification, the heads have been sectioned and stained with Gill's hematoxylin or a silver impregnation method. Selected sections were stained with the peroxidase-anti-peroxidase immunohistochemical method for the demonstration of the olfactory marker protein (OMP).

Lesion to the olfactory system, such as total or partial bulbectomy, induces extensive degeneration of the mature olfactory neurons, ensued by active neurogenesis which leads to the reconstitution of the neuronal population. During and following this process of reconstitution, we have observed in the lamina propria clusters of olfactory neural elements streaming away from the olfactory neuroepithelium. The clusters have different sizes; they can be connected with the neuroepithelium or located deeply in the lamina propria, often adjacent to olfactory nerve bundles. On occasion clusters of olfactory neurons are also present intracranially along nerve bundles which have crossed the lamina cribrosa. Only a limited number of morphologically identified olfactory elements contain OMP; this may suggest a relatively young stage of differentiation of the neurons at any given time or an abnormal OMP methabolism. Serial sections have been routinely used to rule out the possibility that, due to the complex folding of the neuroepithelium lining the nasal cavity, some of the clusters could have been only portions of the neuroepithelium tangentially cut.

Migration of neurons has been observed both in the lesioned and in the unoperated side, possibly indicating a reaction of the system as a whole. Migrating elements can be sporadically observed in normal animals, but the phenomenon never parallels the magnitude reported here. Ultrastructural observations have confirmed the neuronal nature of the extraepithelial elements.

(Supported by NSF BNS-8006803).

295.4 Chemically Active Odorants And Vaporous Protectants As Chemical Probes Of Olfaction. S. P. Fracek, Jr. and R. Schafer. Department of Biological Sciences, North Texas State University, Denton, TX 76203.

The vaporous alkylating agent ethyl bromoacetate (EBA) blocks the ability of the frog olfactory mucosa to respond to odorants other than certain aliphatic amines. Protection from the effects of EBA can be achieved by the simultaneous application of the odorant isoamyl acetate (IAA) (Criswell et al., Science, 210:425, 1980). The work reported here extends these basic findings through the use of several chemically active odorants (odorants which initially produce olfactory responses, but ultimately block those responses). Chemically active odorants tested include diethyl amine (DEA), EBA, ethyl chloroformate (ECF), and ethyl chloroacetate (ECA). Potential protectants which were tested include IAA, para-dichlorobenzene (p-DCB), butyric acid (BA), and 2-mercaptoethanol (2-ME).

Since most of the chemically active odorants caused observable irritation to the olfactory mucosa and adjacent tissues, a preliminary study was done to establish whether a trigeminal nerve response (pain response) might contribute to the electro-olfactogram (EOG) generated by a chemically active odorant. Neither capsaicin in the vapor phase, nor in liquid solution (up to 10^{-2}M in the solvent dimethyl sulfoxide) produced a detectable EOG. Since capsaicin should excite pain fibers maximally, we conclude that there is no substantial trigeminal component in the EOG response to chemically active odorants.

Each of the chemically active odorants, applied as a saturated vapor, rapidly and permanently eliminated EOG responses to itself and to IAA, the reference odorant. No differential effect or specificity of action was observed for any of the chemically active odorants in pre- and post-tests with a variety of odorants of different odor classes. BA, which elicited small surface positive EOGs, had no protective effect against any of the chemically active odorants, while 2-ME produced equivocal results. When applied for long periods (20 seconds), 2-ME initiated a large surface positive DC potential in the mucosa. By contrast, long term exposure of the mucosa to p-DCB produced no DC potential and had no effect on EOG responses. This compound protected against the effects of DEA in a dose-dependant manner, and also showed some protective action against the inhibitory action of ECF, but did not protect against EBA in the concentrations used in this study. IAA protected the mucosa against the effects of both DEA and EBA, but had no protective effect against the inhibitory action of ECF.

295.5 OLFACTORY MARKER PROTEIN: STUDIES OF STRUCTURE AND PRIMARY SEQUENCE. W. Sydor*, Z. Teitelbaum*, R. Blacher*, W. Leung*, Y-C. E. Pan*, L. Brink*, and F. L. Margolis. (SPON: R. Wurzburger) Dept. Physiolog. Chem. & Pharmacol., Roche Institute of Molecular Biology and Dept. Molec. Genetics, Roche Research Center, Nutley, N. J. 07110.

Olfactory marker protein (OMP) is a major acidic, 20K Dalton soluble protein uniquely present in the olfactory mucosa, bulb and nerve of many vertebrate species. It is absent from other brain regions and non-neural tissues. Immunocytochemical studies have demonstrated it to be present solely in the primary olfactory neurons up to and including their terminations in the olfactory bulb. OMP has been purified and is apparently homogeneous on polyacrylamide gel electrophoresis (PAGE). OMP isolated from the mouse and rat have similar, but not identical, amino acid compositions, both lack cysteine and are cross-reactive immunologically. The dynamics of OMP concentrations in olfactory receptor neurons have been correlated to the maturation of these cells. This protein has a relatively long half life in vivo and is transported at a slow rate from the cell bodies to their terminals in the olfactory bulb. However, little is known about its function. The major purpose of this study is to gain additional insight into the primary structure of OMP with subsequent identification of function by homology with the known amino acid sequences of other proteins. Reverse-phase (RP) HPLC of mouse and rat OMP (homogeneous by PAGE) revealed that each is composed of two closely related but clearly separable components, α and β. The relative proportions of α and β in the mouse and rat are 1:2 and 1:13 respectively. Both forms in the mouse and rat react with antisera raised against the PAGE-homogeneous OMP and have similar, but not identical, amino acid compositions. Since the OMP behaves as a monomeric protein, the significance of these two forms is not apparent.

Preliminary studies indicate that both the mouse and rat OMP molecules are blocked at the amino terminal implying post translational modification. Since direct amino acid sequencing of the intact OMP was precluded, HPLC-purified trypsin has been used to generate sequenceable peptides from rat OMP. The number of separable fragments obtained by RP-HPLC of this digest correlates well with the number of Arg + Lys residues in the molecule as determined by amino acid analysis. These tryptic fragments were purified to homogeneity by RP-HPLC and subjected to amino acid analysis and gas phase amino acid sequencing.

The peptide sequences determined to date represent about 25% of the OMP molecule and are being evaluated by computer search to establish structural as well as possible functional homology with known proteins.

295.6 MITRAL CELLS IN THE RABBIT ACCESSORY OLFACTORY BULB: THEIR MORPHOLOGY AND RESPONSES TO LOT STIMULATION. K. Mori* (SPON: Y. SHINODA). Dept. of Physiol., Gunma Univ. Sch. of Med., Maebashi, Gunma, 371, Japan.

Intracellular recordings were made from mitral cells of the rabbit accessory olfactory bulb (AOB). Micropipettes filled with 4% horseradish peroxidase (HRP) in 0.1M Tris buffer containing 0.5M potassium acetate were used to record the spike activity and synaptic potentials. The HRP was injected into the mitral cells by pulses of depolarizing current (5-10 nA) with a duration of 600 msec at a frequency of 0.8 c.p.s. The results were compared with those obtained from mitral cells of the main olfactory bulb (MOB).

In most mitral cells of the AOB, lateral olfactory tract (LOT) stimulation elicited an antidromic action potential followed by an inhibitory postsynaptic potential (IPSP), as is the case with MOB mitral cells. The latency of the antidromic action potential ranged from 4.1 to 30.1 msec (mean 15.2 msec, N=12). The axonal conduction velocity was approximately 0.4-3.1 m/sec which is much slower than that of the MOB mitral cells (about 10 m/sec). In some cases, the onset of the IPSP preceded the antidromic action potential.

Seven HRP injected mitral cells were reconstructed from serial frontal sections of the AOB. Two to three primary dendrites emanated from the soma and projected in various directions in the external plexiform layer (EPL). The primary dendrites branched a few times in the EPL and then made small and dense terminal arborizations with various shapes in several portions of the glomerular layer. This is in contrast with a single, large, and round terminal arborization of the primary dendrite in MOB mitral cells. Secondary dendrites emanated from the proximal portion of the primary dendrites and were distributed in the EPL. The secondary dendrites were fewer in number, thinner in diameter, and much shorter in length than those of the MOB mitral cells. The axon of the AOB mitral cells bifurcated a few times in the granule cell layer to produce thin axon collaterals. As is the case with MOB mitral cells, the projection field of the axon collaterals was spatially separated from the dendritic projection field. The main axon then entered the dorso-medial portion of the LOT.

These preliminary studies show that mitral cells of the AOB and those of the MOB are similar in the responses to the LOT stimulation and projection patterns of the dendrites and axon collaterals. In addition, these studies demonstrated marked differences between them in the details of the morphology and the response properties, which may be related to the differences in the processings of the chemical signals.

295.7 OLFACTORY BULB MITRAL AND TUFTED CELL PROJECTIONS TO THE ANTERIOR OLFACTORY NUCLEUS. J.W. Scott, E. C. Rainer and E. Orona. Department of Anatomy, Emory University, Atlanta, Georgia 30322

Previous studies have shown that the output of the olfactory bulb is topographically organized in the lateral olfactory tract (LOT) and possibly in the anterior olfactory nucleus (AON) (eg. Price and Sprich, J.C.N. 1975; Schoenfeld and Macrides, AChemS V). However, it has not been possible to tell whether terminals in the AON follow the same organization because of difficulties distinguishing fibers of passage. Several retrograde transport studies are consistent with the external tufted cells projecting mainly to rostral structures such as the AON.

We studied the mitral and tufted cell projection to the pars externa (pE) of the AON by small, iontophoretic extracellular injections of horseradish peroxidase into the external plexiform layer (EPL) of the olfactory bulb. These injections labeled the proximal portion of the mitral and tufted cell axons so that they could frequently be followed in the LOT past the AON. In 18 of 23 EPL injections, short branches were seen to leave the LOT and terminate within layer I of the AON pE. These branches usually subdivided several times but these processes were confined to a small portion of the pE. Branches from axons over more caudal part of the AON or anterior piriform cortex could also be seen, but they were usually longer and could often be followed to the olfactory tubercle. Thus the terminal endings over the pE appear to preserve the topography of the LOT while the termination of the more caudal branches is more difficult to determine.

The small size of the iontophoretic injections has enabled us to make some conclusions about the cell types that project terminal branches into the pE. Injections into the superficial EPL labeled tufted cells or those mitral cells with superficial basal dendrites (Orona et al. Anat. Rec. 1983). These injections produced the most abundant terminal branching in the pE. However injections in the deep EPL that labeled mitral cells with deep basal dendrites also produced labeling of terminal branches in pE. By axonal reconstruction, it was possible to trace branches from all types of output cells to the terminal branches in pE.

We conclude that there is spatial organization in the output from the olfactory bulb to the pE of the AON, and that all types of output cells contribute to that projection. Along with the report of Davis et al. (J.C.N. 1981) that efferents from the pE to the contralateral olfactory bulb are topographically organized, this suggests a reciprocal interaction of regional parts of the olfactory bulb.

Supported by NSF grant BNS-8102175

295.8 EVIDENCE SUGGESTING THAT DIAGONAL BAND AFFERENTS TO THE MAIN OLFACTORY BULB ARE CHOLINERGIC IN THE RAT. S. Van Ooteghem*, M. T. Shipley, M. S. Sanders* and S. Schumacher* (Spon: S. H. Bryant). Depart. of Anat. and Cell Biol., Col. of Med., Univ. of Cincinnati, OH 45267.

An afferent pathway from the nucleus of the diagonal band (DB) to the main olfactory bulb (MOB) has been described in several species. Neurons of DB provide a major, and possibly, the sole source of cholinergic input to the MOB in the hamster (Macrides, et al., J.C.N., 1981). However, it has not been established whether all, or even a significant proportion of DB→MOB projection neurons are cholinergic. It has been difficult to answer this question, partly because the neuropil in the region of the DB stains so densely in AChE histochemical material that it cannot be resolved from the background.

To address this question, adult, male Sprague Dawley rats were given a sublethal dose (1.8 mg/Kg, I.M.) of diisopropyl florophosphate (DFP) four hours before sacrifice to temporarily deplete AChE levels (Butcher, et al., J. Neural Transm., 1975). The brains of these animals were examined for AChE using a modification of Koelle's method.

The neuropil of DFP-treated animals was nearly devoid of staining. Individual AChE-positive neurons and their proximal dendrites stood out in Golgi-like fashion. With the dose and survival time used, staining was absent in fine distal processes. This dramatic improvement in the visibility of AChE+ somata is probably due to the fact that it takes a few hours for newly synthesized AChE to migrate from somata into distal processes. This technique most likely results in an underestimation of the number of AChE+ neurons because at survival times which optimize the resolution of single cells many neighboring large neurons are still only weakly positive.

Following injections of WGA-HRP in MOB, heavy labeling of most large neurons in the DB was observed. The similarity between the size, number and appearance of these labeled neurons and the AChE+ neurons of DFP-treated rats was striking.

These results suggest that most, if not all, DB→MOB projection neurons are cholinergic in the rat. To firmly establish this point it will be necessary to demonstrate retrograde labeling and AChE and/or CAT activity simultaneously. Experiments designed to accomplish this are in progress.

MOB appears devoid of intrinsic cholinergic neurons. The precise delineation of the DB→MOB projection is therefore important since it may provide an excellent model for studies of development, function and aging of an extrinsic cholinergic pathway to a "simple" cortical structure.

Supported by NIH grants NS-18490, NS-19730 and USAMRDC DAMD17-82-C-2272.

295.9 THE ROLE OF NORADRENALIN IN OLFACTORY RECOGNITION. A. E. Rosser* and E. B. Keverne* (SPON: European Neuroscience Association). Department of Anatomy, University of Cambridge, Cambridge, U.K.

Noradrenergic mechanisms have been implicated in enhancing signal to noise ratio in the visual, auditory and somaesthetic senses, and have recently been proposed to facilitate transmission in the olfactory sense (Jahr, C.E., Nicoll, R.A. Nature, 297, 227-229, 1982). The purpose of this study is to examine whether there is a role for noradrenalin (NA) in the recognition of different strains of mice, and the model chosen is the olfactory block to pregnancy. This effect is a pheromenal one mediated via the accessory olfactory system and involves recognition and memory of the stud male since only male pheromones of a different strain have this capacity.

Centrifugal NA fibers reach the olfactory bulbs via the medial olfactory striae. Bilateral injections of 6-hydroxy-dopamine (6OHDA) into the striae 6 days prior to mating, effectively depletes NA and results in a failure to recognise the stud male, since his pheromones now block his own pregnancy (Keverne, E.B., de la Riva, C., Nature, 296, 148-150, 1982).

To investigate whether NA is required in the recall process of the memory, it was necessary to make 6OHDA lesions into the medial olfactory striae after memory formation at mating, with subsequent re-introduction of the stud. In this experiment, a model of pseudopregnancy was used to avoid the problem of implantation on day 4 in pregnancy, after which the olfactory block to pregnancy cannot be demonstrated, and permit re-introduction of the male at a later time when the lesion was complete. Re-introduction of the stud in NA depleted animals did not produce an early return to estrus from pseudopregnancy when compared with control groups. Hence, NA is required for formation, but not recall of this memory.

In order to identify a possible component of mating responsible for causing NA release, stimulation of the cervix, which occurs during mating, was performed mechanically on females and NA turnover in the olfactory bulbs was examined. A significantly higher rate of turnover was found at 1, 2 and 3 hours following stimulation, but not at 12, 48 or 96 hours. This parallels our finding that the minimum time of exposure of females to male pheromones following mating for the memory to be formed, lies between 3 and 4½ hours. In those females allowed to remain with the male for 3 hours post coitus, only 30% remained pregnant on re-introduction of the stud the following day, whereas in females exposed for 4½ hours, 86% remained pregnant.

It would therefore appear that the NA projection to the accessory olfactory bulbs is activated following cervical stimulation during mating and remains so over the period during which memory formation occurs.

295.10 HIGH DENSITY PERIPHERAL-TYPE BENZODIAZEPINE RECEPTORS ON PRIMARY OLFACTORY AFFERENTS. Kenneth M.M. Murphy, Robert R.H. Anholt*, Gregory E. Mack* and Solomon H. Snyder. Johns Hopkins University, Depts. of Neuroscience, Pharmacology and Psychiatry, School of Medicine, Baltimore, Maryland 21205.

Use of Ro5-4864, a ligand selective for peripheral-type benzodiazepine receptors, demonstrates the presence of a high density of peripheral-type benzodiazepine receptors in the glomerular layer of the olfactory bulb and a diffuse distribution throughout the rest of the brain probably on glial cells. We present evidence that binding sites for [^{3}H]-Ro5-4864 in the olfactory bulb are localized on afferents originating from olfactory receptor cells in the nasal epithelium. This notion is supported by the observation that destruction of the nasal mucosa by intranasal irrigation with $ZnSO_4$ leads to a significant reduction in the density of Ro5-4864 binding sites in the olfactory bulbs. Furthermore, binding studies to membranes obtained from nasal epithelium and autoradiography on cryostat sections of the nose reveal a high density of peripheral-type benzodiazepine binding sites (approximately 4 nmoles/g protein). Displacement studies show a similar pharmacology between these receptors in the nose, the olfactory bulb, the brain and the kidney. Ligands, which are selective for the central-type benzodiazepine receptor, such as clonazepam, do not bind to nasal epithelium. The presence of peripheral-type benzodiazepine receptors on nasal membranes and the distinct pharmacology characteristic of the peripheral-type benzodiazepine receptor are found in several mammalian species, namely mouse, rat, guinea pig, cat and dog. We speculate that the high density of peripheral-type benzodiazepine receptors on primary olfactory afferents may be related to the continuous turn-over of these olfactory receptor cells and the great regenerative capacity of the nasal epithelium from neurogenic stem cells.

295.11 MAMMALIAN OLFACTORY SYSTEM - A FIVE-FOLD COMPARATIVE MODEL. Pauline Jirik-Babb and Richard S. Babb. Dept. of Neuroscience, Albert Einstein College of Medicine, The Bronx, NY, 10461, and Iona College, New Rochelle, NY, 10801.

A five-fold model of the mammalian olfactory system is presented viewed from an evolutionary perspective, and based on anatomical, physiological and behavioral comparative data. The mammalian olfactory system is considered to have developed phylogenetically in five stages corresponding to prechordate, chordate, vertebrate, tetrapod and mammalian evolution.

(1) Chemical irritants act on free nerve endings of the subdural nerve plexus of prechordates (Bullock, 1940). This general tactile sense is thought to be homologous with the free nerve endings found in the mucous membranes of the nasal cavity which are supplied by the ophthalmic branch of the trigeminal nerve. (2) The terminal nerve which ends in the preoptic region of the central nervous system, is found in all chordates. The sensory endings of the terminal nerve of male fish have been shown to be sensitive to the release of sex pheremones of females, and to mediate sperm release (Demski and Northcutt, 1983). (3) The accessory (vomeronasal) olfactory epithelium which has been hypothesized to have evolved in vertebrates (Broman, 1920) and to be sensitive to odors in an aquatic environment, projects via the accessory bulb to the amygdala. (4) The main olfactory system has been theorized (Babb and Jirik-Babb, 1983) to have differentiated from the accessory system during the phylogenesis of tetrapods, and to be specialized for the detection of airborne odors. (5) Cells of the septal organ have been illustrated by Graziadei (1976) and are similar to main olfactory epithelial cells. Singh*, et al. (1976) have shown that an intact main olfactory epithelium, which would include the septal organ, is necessary for suckling. Further Shepherd (private communication, 1983) has suggested that the septal organ may project to the modified glomerular subregion of the main olfactory bulb which has been shown to be active during suckling (Teicher, et al., 1980). Singh* and Hofer (1978) suggest that a putative pheremone secreted by apocrine glands in the mammary region of the rat mother is instrumental in orientation and attachment to the nipple by rat pups. Thus the septal organ and the modified glomerular subregion may form a unique mammalian olfactory system which detects a putative pheremone released by the mother enabling neonates to orient and attach to the nipple.

The comparative evidence discussed above leads to a modification of the dual olfactory system idea of Kappers, et al. (1936) and suggests a five-fold olfactory system based on evolutionary ideas. The individual olfactory subsystems have developed phylogenetically in an hierarchial manner and operate separately and in parallel.

* now Jirik-Babb

295.12 DISTRIBUTION OF TASTE AND TACTILE NERVES IN THE FUNGIFORM PAPILLA OF THE HAMSTER. M. Whitehead and C. Beeman*. Dept. of Oral Biology, University of Connecticut School of Dental Medicine, Farmington, CT 06032

Fungiform papillae of the rodent tongue are heavily innervated by both the chorda tympani and the lingual branch of the trigeminal nerve (Beidler, Olfaction and Taste, 1965; Miller, J. comp. Neurol., 1974). Chorda tympani fibers mediate taste and their endings presumably are restricted to the taste bud. Lingual nerve fibers mediate tactile and temperature sensitivity but their site of termination has not been defined. The distribution of lingual fibers in hamster fungiform papillae was examined by electron microscopy. The chorda tympani was sectioned and these fibers were allowed to degenerate for one or three days prior to perfusion of the animals with a buffered fixative containing mixed aldehydes.

Terminals of the lingual nerve were concentrated near the surface of the fungiform papillae within the non-taste, squamous epithelium which surrounds the apical region of the taste bud and the taste pore. The endings are dilated and intimately apposed to the cells of the superficial layers of the stratum spinosum, just beneath the stratum corneum. This latter cornified layer is thinner in this region than on the sides of the papillae or elsewhere on the tongue. Ubiquitous collections of tonofilaments occur in the epithelial cytoplasm encircling the nerve endings. While lingual terminals were readily observed in perigemmal locations, the taste buds were virtually devoid of intact nerves one day following destruction of the chorda tympani. Remnants of chorda tympani fibers including myelin figures were restricted to the taste buds and cores of the papillae. The cells of denervated taste buds at one and especially at three days post-lesion exhibited a dramatic and rapid transformation--the cells proliferated microvilli and spaces between the cells enlarged. However, degeneration of cells in denervated taste buds was not seen.

These data document a segregation of chorda tympani fibers in the taste bud, and lingual nerve fibers in a discrete perigemmal area of the fungiform papilla. The lingual nerve-epithelial arrangement and superficial location near the least cornified area of the tongue may be well suited for discriminative mechanoreception. Thus, the apical surface of individual fungiform papillae could be a primary site of interaction of both taste and tactile aspects of oral stimuli with receptors.

Supported by NIH Grant NS16993 and the CT. Research Foundation.

295.13 Transganglionic transport of HRP from the circumvallate papilla to the brainstem in rats. R.M. Bradley, C.M. Mistretta, C.A. Bates and H.P. Killackey*. Oral Biol., Dent., Univ. Michigan, Ann Arbor MI 48109 and Psychobiology, U.C. Irvine, Irvine CA 92717.

Gustatory projections to the brainstem have been studied with transganglionic transport of HRP applied to cut, central ends of taste nerves. We now demonstrate that these projections can be traced after injecting HRP directly into taste papillae. Wheat germ agglutinen HRP (0.5 - 1.0 µl, 5%) was injected into the single circumvallate papilla in 8 rats aged 15 to 30 days. After 48 hours rats were sacrificed; brainstem and tongues were prepared for HRP histochemistry, using tetramethyl benzidine as a chromagen. HRP label in tongue sections was limited to the papilla and immediate surround. In the medulla both sensory and motor fibers of the glossopharyngeal nerve and terminal fields in the solitary tract were labelled bilaterally, as far caudally as the rostral pole of the hypoglossal motor nucleus. In addition a discrete group of small cells was labelled immediately ventral to the solitary nucleus,just rostral to the point of entry of the glossopharyngeal nerve. We refer to these cells as the dorsal motor nucleus of IX. Also some larger cells were labelled in the rostral part of nucleus ambiguus.

These findings were compared to central projections after placing HRP crystals on the cut, central end of the lingual branch of the glossopharyngeal nerve in 8 rats. HRP label in the solitary tract was much more intense and more cells were labelled in nucleus ambiguus. There was no difference in extent of label in dorsal motor nucleus of IX. In two other rats, HRP crystals were applied to the cut central end of the glossopharyngeal nerve on one side and the vagus on the other. Labelled central projections of the vagus nerve were similar to those described recently by Kalia & Sullivan (J. Comp. Neurol. 211:248,'82). Sensory fibers of the vagus extended as far rostrally as the point of entry of the glossopharyngeal nerve. By labelling glossopharyngeal and vagus in the same rat, it became apparent that the dorsal motor nucleus of IX is an extension of the dorsal motor vagal nucleus. In summary, papilla injections of HRP label projections of sensory and motor, glossopharyngeal nerve fibers which innervate the circumvallate papilla. The sensory fibers probably innervate taste buds and the motor fibers, von Ebner's glands. In the brainstem, the close apposition of sensory input and secretomotor output to the circumvallate papilla suggests a possible reflex gustatory control of the secretion of von Ebner's glands.

(Supported by grants N.I.H. DE05728 and N.S.F. BNS80-15737 to RMB and CMM, N.S.F. BNS81-20658 HPK, and N.I.H. Fellowship MH08610 to CAB.)

295.14 EFFECT OF AMILORIDE ON CHORDA TYMPANI RESPONSES TO SALTS. J.H. Teeter*, W.L. Silver, and J.G. Brand*. Monell Chemical Senses Center, Philadelphia, PA 19104.

The diuretic drug amiloride is a specific and reversible inhibitor of sodium transport in a variety of epithelial and cellular transport systems (Benos, Am. J. Physiol., 11:C145, 1982 for review). Amiloride has been found to reduce Na^+ flux across in vitro preparations of dorsal lingual epithelium of the dog (DeSimone et al., Science, 214:1039, 1981) and to reduce the perceived intensity of NaCl, Na_2SO_4 and sucrose, but not KCl, K_2SO_4 or $CaCl_2$, in humans (Schiffman and Lockhead, Neurosci. Abstr., 7:12, 1982). These results suggest that an amiloride-sensitive Na^+ flux may be important in NaCl and sucrose taste reception.

We examined the effect of amiloride on integrated chorda tympani nerve responses of the gerbil to various concentrations of NaCl, LiCl and KCl. Ascending concentrations (0.001-1 M) of each salt were applied to the tongue (1 ml aliquots) and the integrated whole nerve responses recorded. Stimuli were superimposed upon a continuous flow of deionized water washing the tongue. The tongue was then washed for 2 min with 5×10^{-4} M amiloride and the concentration-response relations for each salt were again determined. Finally, the tongue was washed for 60 min with deionized water and the responses to 1 M concentrations of the salts were recorded periodically during this time.

Amiloride treatment of the tongue resulted in a 60-90% reduction in amplitude of the responses to NaCl and LiCl at concentrations greater than 10-50 mM. The responses evoked by lower concentrations of NaCl and LiCl were not reduced. Inhibition was never complete. The amplitude of the whole nerve response was reduced to about the same level regardless of the stimulus concentration. The responses to KCl were either not affected by amiloride or they were reduced to a much smaller extent than the responses to the other salts. Responses to 1 M concentrations of NaCl and LiCl started to recover several minutes after the deionized water rinse was begun. Complete recovery, however, was not observed even after 60 min. These results are consistent with the hypothesis that part of the taste response to NaCl and LiCl is mediated by amiloride-sensitive channels in the apical membranes of the taste cells.

Supported by NIH grant NS15804 and a grant from the Whitehall Foundation.

295.15 IS THE NERVUS TERMINALIS A TWO COMPONENT NERVE? Celeste R. Wirsig Dept. Neuroscience, University of Florida, Gainesville FLA 32610

The Nervus Terminalis is a candidate for a new chemosensory nerve. In mammals its fibers arise in the nasal and vomeronasal epithelium and course back between the olfactory bulbs to terminate in several forebrain areas. One peculiar feature of this nerve is the presence of ganglion cells along its entire course. A larger cell grouping between the bulbs comprises the Ganglion Terminale (GT). I previously reported (Wirsig,1983) that, in the 5-day-old hamster, the GT cells are AChE-positive and are distributed along the vomeronasal nerve and the ventromedial surface of the bulb and anterior olfactory nucleus. In the present study the location of the AChE positive GT cells was mapped in the adult hamster to compare the distribution pattern of the cells as development occurs. Tissue was stained with the Tsuji method for demonstrating AChE. AChE positive cells follow a very similar course in the 5-day-old and adult hamster. In the 5-day-old, AChE positive cells are most densely packed at the level of the rostral bulb, but in the adult these cells are more evenly distributed along the nerve. The number of cells is similar in both ages (ranging from 65-120), suggesting that the cells are being displaced caudally during development. This would suggest a periperal site of origin, possibly the olfactory placode. Brookover & Jackson (1911) believed that the NT cells arise from the olfactory placode, but it has also been contended that some of the cells migrate out of the forebrain or from the neural crest during development. Therefore there may be two populations of cells comprising the nerve. Many of the early investigators (Johnston,1914; Larsell, 1918; Pearson, 1941) speculated that the nerve possesses both a sensory and an autonomic component, but to date there is no concrete evidence for this. Recent studies have shown that the GT cells contain LHRH (Schwanzel-Fukuda, 1980) and AChE (Wirsig,1983) supporting the notion of two separate populations of NT cells. Since the forebrain contains a rich supply of LHRH positive cells it might well be the source of the NT component. To compare the distribution of AChE positive and LHRH positive cells during development,immunocytochemical studies are underway in mature and immature hamsters. The possibility of the coexistence of AChE and LHRH in the cells will also be examined.

Supported by NS 13516 to CM Leonard, MH 15737-02 to CR Wirsig and Sigma Xi Grant-in-Aid to CR Wirsig.

Brookover & Jackson 1911 JCN 21: 237.
Johnston 1914 Anat. Rec. 8: 185.
Larsell 1918 JCN 30:3.
Pearson 1941 JCN 75: 39.
Schwanzel-Fukuda 1980 JCN 191: 213.
Wirsig 1983 Assoc. Chemoreception Science. Abstract.

295.16 DECREASED GUSTATORY NEURAL DISCHARGES TO SALTS IN THE ADRENALECTOMIZED RAT. Therese Kosten* & Robert J. Contreras. Yale University, Dept. of Psychology, New Haven, CT 06520.

Sodium deficiency in the rat leads to salt appetite and to increased consumption of salt solutions; the normal salt preference curve is altered such that there is greater acceptance of hypertonic, normally aversive solutions. This behavior is innate, occurs rapidly and is relatively specific to sodium solutions. The initiation, maintenance and satiation of the behavior is mediated, in part, through the gustatory system. The classic model for the study of salt appetite is the adrenalectomized rat; sodium deficiency is due to the removal of the major component of the mechanism that maintains sodium homeostasis. Using this preparation, we present evidence for a decrease in peripheral neural sensitivity to salt solutions.

Whole nerve chorda tympani recordings were taken from adrenalectomized rats maintained on 0.3 M NaCl solution and from control rats. The taste stimuli included a series of NaCl solutions and two concentrations each of LiCl, KCl, HCl, and quinine HCl which were all presented after a rinse of deionized water. The peak neural discharge and a measure of tonic discharge were analyzed relative to the response to 0.001 M HCl to facilitate comparison across animals. In additions, the data were analyzed, using Beidler's biophysical model of stimulus-receptor interaction, to provide evidence for receptor mechanisms. As a control for generalized decreases in sensory sensitivity, recordings from the auriculotemporal nerve in response to a series of tactile stimuli given to the ear were also performed in these two sets of animals.

The data show that adrenalectomized rats have a decreased response to suprathreshold concentrations of NaCl ($F(7,63) = 7.16$; $p < .05$) and LiCl ($F(1,9) = 8.98$; $p < .05$) but not to KCl and quinine HCl. No differences were seen in these animals' neural responses to the tactile stimuli. Decreased neural activity may signal a lower intensity of salt solution thus leading to greater acceptance of the higher concentrations and ultimately to greater intake. The peripheral alterations may be part of a short-term control system of salt intake. The changes are fairly specific indicating a decrease in presumed salt receptor activity or in the number of receptors functioning perhaps due to disuse.

This research was supported by NIH, NHLBI Grant HL-28952.

295.17 TASTE RESPONSES OF PARABRACHIAL UNITS TO NACL AND SACCHARIN IN RATS THAT WERE PRETRAINED TO AVOID SACCHARIN. P. Di Lorenzo* and J. Garcia* (SPON. P. Lasiter). Dept. of Psychology, University of California at Los Angeles, 90024.

Taste aversion conditioning produces an hedonic shift that is associated with a gustatory CS, i.e. a previously preferred tastant will subsequently be avoided. In addition, this learning process alters the hedonic properties of other tastants according to their similarity to the CS. In effect, after the initial contact with a stimulus, the conditioned stimulus animal is required to perform more precise discriminations of gustatory quality before further decisions about consumption can be made. Naive animals, in contrast, would presumably not require as much precise information about taste quality before making such decisions.

Previous studies have suggested that behavioral/hedonic decisions may be reflected in the temporal patterns of taste responses in the parabrachial nucleus of the pons (PbN). The initial phasic excitatory component has been related to hedonic evaluations while the longer-lasting tonic component may reflect the analysis of gustatory quality. It was hypothesized, therefore, that the hedonic changes that are produced by taste aversion conditioning may be reflected in the temporal patterns of gustatory responses in the PbN. The present study was designed to study the nature of parabrachial responses to NaSaccharin (SAC) and NaCl in flaxedilized rats that were pretrained to avoid SAC in a behavioral paradigm. These responses were compared to responses in naive rats.

Preliminary analysis of both single and multiunit records suggests that the responses to SAC in pretrained rats are indistinguishable from those in naive rats; however, the NaCl responses show distinct temporal patterns in each group. In naive rats, the response to NaCl is most commonly characterized by both phasic and tonic components. In contrast, the NaCl responses in pretrained rats are most often predominantly tonic in nature.

The increased incidence of tonic excitatory responses to NaCl in pretrained rats may reflect an amplification of the neuronal code for NaCl in the PbN. In contrast to elements that show predominantly phasic gustatory responses, units that respond with tonic excitatory responses may provide more spike activity with which to encode taste quality. This would increase the signal to noise ratio of the neural response and may facilitate the additional gustatory discriminations that are required by conditioned animals.

Supported by the following: National Institute of Health Grant NS11618 & Program Project Grant HD05958. P. Di Lorenzo supported by NIMH Research Service Award MN15795.

295.18 EVIDENCE FOR MEMBRANE POTENTIAL CONTROL OF CHEMORECEPTION IN PARAMECIUM TETRAURELIA. S. Schulz*, M. Denaro* and J. Van Houten. Dept. of Zoology, Univ. of Vermont, Burlington, VT 05405.

Paramecia are ciliated unicells that detect soluble chemicals in their environment and swim to accumulate or disperse in response. Van Houten has developed an hypothesis of membrane potential (Vm) control of chemoresponse (Van Houten, J., J. Comp. Physiol., 127: 167, 1978) that takes into account electrical control of ciliary beating (hence swimming) and the two swimming behavioral mechanisms by which paramecia accumulate and disperse. This hypothesis has been used to predict transduction of chemical cues into characteristic Vm changes in attractants and repellents relative to controls (Van Houten, J., Science, 204: 1100, 1979). In further tests of this hypothesis, we have predicted a) accumulation and dispersal based on measured Vms and b) defective Vm changes for some of our chemoresponse mutants.

a) The Vm hypothesis was developed to account for responses to organic compounds. However, if the hypothesis were correct, one should be able to manipulate Vm with external inorganic cations and predictably cause accumulation or dispersal. We used a series of K and Na buffers, in which Paramecium Vm ranged from -18 to -43 mV, for measuring accumulation and dispersal behavior in situations where differences in Vm in test and control solutions are known. We found good quantitative and qualitative agreement with predicted behavioral response based on differences in Vm alone.

b) If change in Vm with exposure to attractant or repellent solution were an essential step in chemosensory transduction, we would expect to find among our chemoresponse mutants cells blocked in this step or earlier in the pathway. We predicted that a mutant defective in an early step, such as binding the attractant folate, would fail to complete one of the next steps in the pathway, namely change of Vm. Normal cells showed accumulation in K_2folate (I_{che}= 0.76 $\pm$ 0.06), saturable binding of ^{3}H-folate (K_d = 35 μM), and characteristic hyperpolarization in attractant folate relative to control chloride (Cl) (Van Houten, J., Science, 204: 1100, 1979). Mutant d4-534 showed reduced chemoresponse (I_{che}= 0.58 $\pm$ 0.13) (DiNallo, M., et al., Genetics, 102: 149, 1982), greatly reduced binding with no measurable Kd, and greatly reduced difference between Vm in folate and Cl. The failure to normally change Vm in folate could account for the mutant phenotype and fulfills our expectations for a mutant lesion early in the pathway. Other mutants, such as mtx^6, while not accumulating in folate (I_{che}= 0.47 $\pm$ 0.09), showed extensive binding and normal Vm changes, and were expected to have blocks elsewhere in the chemosensory transduction pathway.

This work was supported in part by NSF 15350 and NIH GM29045.

295.19 BEHAVIORAL GUSTATORY RESPONSES OF PERIPLANETA AMERICANA TO D&L AMINO ACIDS. Dorothy Sugarman* and William Jakinovich, Jr. Lehman College, Dept. Biological Science, CUNY, Bronx, N.Y. 10468

This study explores behavioral gustatory responses in the adult cockroach, Periplaneta americana, to amino acids. Insects were mounted and tested using a modified Frings (1946) method. Results include the following:

1. Roaches responded positively, in varying degrees, to 23 L-amino acids dissolved in deionized water. However, responses were negative to the 13 D-amino acids tested, with exception of D-Trp and D-DOPA.

2. Two concentration series of all the L-amino acids were prepared, one in deionized water and one in 0.1M Na-Phosphate buffer, pH 7.0. In general, overall increased response was noted to buffered solutions.

3. Most dramatic increase was seen in buffered responses to six amino acids with non-polar R-groups, i.e. Val, Leu, Ile, Meth, Phe and Trp. Moreover, all six exhibited similar response patterns in water and in buffer, with greatest preference shown for 0.01M concentration (.005M for Trp).

4. Only Proline and Hydroxy-proline failed to show any increased response to buffered solutions but reflected the same response pattern throughout.

5. While roach response to selected L-amino acids in NaCl and Na-buffered solutions showed enhancement, solutions of K-buffer or KCl did not. It was concluded that increased response was due to some type of sodium ion involvement.

Supported by a grant from the National Institutes of Health.

295.20 MIXTURE SUPPRESSION: NEURAL INTEGRATION OF A COMPLEX ODOR. Barry W. Ache* and Charles D. Derby. C. V. Whitney Laboratory, University of Florida, Rt. 1, Box 121, St. Augustine, FL 32084

Low molecular weight organic compounds are behaviorally-relevant stimuli for many aquatic organisms. These compounds usually work in mixtures, which can be defined, both quantitatively and qualitatively, from naturally-occurring sources. In the present study, the intensity of neural activity evoked in high-order interneurons descending the circumesophageal connectives of the spiny lobster (Hamilton, K. A. and Ache, B. W., J. Comp. Physiol. 150: 129, 1983) is used as an assay (1) to identify the stimulatory components of a crab muscle tissue extract and (2) to determine the type of contribution each component makes to the mixture based on a first-order, additive model. A synthetic mixture of 31 chemicals is adequate to account for the stimulatory capacity of the natural extract. Eleven of these components do not contribute to the mixture. The remaining 20 components (13 amino acids, 3 organic bases, 1 organic acid and 3 nucleotides) are contributory and all exhibit potential hypoaddition (suppression) in mixture. None exhibit hyperaddition (synergism). The contributory components, however, vary in their capacity to evoke activity when presented singly, from taurine which by itself elicits activity not significantly different from that elicited by the complete mixture, to 7 amino acids and homarine which are subthreshold for activation at their concentration in the mixture. Combining the compounds into sub-mixtures by type of action and reassaying confirms the mixture consists of 12 interactive and stimulatory components, 8 interactive but non-stimulatory components, and 11 non-contributory components. Recording from both extrinsic neurons of the olfactory lobe (first-order interneurons) and receptors indicates at least some of the observed mixture suppression occurs very early in the olfactory pathway. These findings set the stage for a detailed analysis of the loci and cellular mechanism(s) of mixture suppression in olfaction. They also stand in contrast to behavioral evidence, from both crustaceans and fish, that aquatic chemostimulants synergize in mixture.

295.21 ODOR QUALITY CODING AT THREE NEURONAL LEVELS IN A GLOMERULAR-TYPE BRAIN. Charles D. Derby, Barry W. Ache* and Kathyrn A. Hamilton. C. V. Whitney Laboratory, University of Florida, St. Augustine, FL 32084

To understand odor quality coding, it is necessary to describe patterns of neuronal convergence. We are using a simple preparation to decipher coding mechanisms at three neuronal levels: olfactory receptor cells, low-order interneurons, and brain output interneurons. This preparation, the brain and olfactory organs of the spiny lobster, has many parallels with the vertebrate olfactory pathway; e.g. ciliated bipolar, primary receptor cells with peripheral somata, and receptor axons that synapse onto interneurons in a glomerular-type neuropile. Yet its relative simplicity makes the problem of understanding coding mechanisms more tractable. We are using a defined mixture and its components to describe the specificity (breadth of responsiveness) and across-neuron response patterns of receptor cells, interneurons, and brain output interneurons. Most receptor cells responded to only 1 of 8 components tested, and the response to this compound equaled the response to the mixture. Cells with slightly broader response spectra were also found. Overall, receptor cells showed very low breadth of responsiveness and across-neuron correlations for pairs of stimuli. Low-order interneurons showed much greater breadth of responsiveness, with most responding to at least several of the 8 individual components tested. Although there are some differences in response spectra of interneurons grouped according to receptive field- or modality-sensitivity, the greater homogeneity of these cells relative to receptor cells is reflected in their much higher values for breadth of responsiveness and across-neuron correlations for pairs of stimuli. Compared to low-order interneurons, brain output interneurons have greater breadth of responsiveness but, somewhat surprisingly, lower across-neuron correlations. There is also evidence for interaction (suppression) of components in the mixture at the receptor and both interneuronal levels. These data suggest that extensive convergence of narrowly-tuned peripheral channels onto low-order interneurons can occur at early synaptic levels, and that further convergence can modify the olfactory messages by the level of the brain output interneurons.

295.22 MIXTURE SUPPRESSION OF PRIMARY CHEMORECEPTOR RESPONSES: NEUROPHYSIOLOGICAL EVIDENCE IN TAURINE SENSITIVE CELLS. Richard A. Gleeson* and Barry W. Ache* (SPON: R. Cagan), Monell Chemical Senses Center and C. V. Whitney Laboratory, University of Florida, Rt. 1, Box 121, St. Augustine, FL 32084

Under natural conditions, both the olfactory and gustatory systems are exposed to complex mixtures of chemical stimuli. Although a number of psychophysical studies (Bartoshuk, Physiol. Behav. 14: 643-649, 1975) have begun to define the relationships between sensations evoked by pure compounds and mixtures, only a few workers have addressed this question neurophysiologically (Hyman and Frank, J. Gen. Physiol. 76: 125-142 and 143-173, 1980). In the present study, single unit recordings from chemoreceptors on the olfactory organ (antennule) of the spiny lobster revealed a subpopulation of taurine sensitive cells (47% of the cells examined) whose response to taurine is inhibited by certain amino acids. A synthetic mixture of 22 amino acids, which mimics the composition of a natural food stimulus (crab muscle tissue) and itself contains taurine, totally and reversibly blocks the taurine response of this group of receptor cells. An analysis of the contribution to this suppression by the six major components (based on concentration) in the mixture revealed partial or complete inhibitory activity by five of the compounds. In a sample population of the inhibited cells, mean percent suppression of the taurine response was 99 for glycine, 96 for L-arginine and L-glutamate, 60 for L-alanine and 42 for L-proline. The nature of this inhibition is currently under study. These results suggest that the processing of chemical information in quality and/or intensity coding of natural stimulus mixtures is tempered by interactions of the components at the receptor-cell level.

295.23 RESPONSE SPECTRA OF THE ANTENNULAR CHEMORECEPTORS IN THE AMERICAN LOBSTER, HOMARUS AMERICANUS. B. R. Johnson* and J. Atema. Boston Univ. Marine Program, Mar. Biol. Lab., Woods Hole, MA 02543.

The lateral filament of the lobster's antennular flagellum is a chemoreceptor organ (cf. smell) providing information necessary for efficient orientation to odor sources (Reeder, P. and Ache, B. W., Anim. Behav., 28:831, 1980 and Devine, D. V. and Atema, J. Biol. Bull., 163:144, 1982). Chemoreceptors of the lateral filament are sensitive to a variety of low molecular weight compounds, particularly amino acids, which elicit feeding activity but little is known about the response spectra of the individual chemoreceptors. Dactyl chemoreceptors (cf. taste) in this animal showed remarkable single compound specificity (Derby, C. D. and Atema, J. J. Comp. Physiol., 146:181, 1982). In the present study we surveyed the response specificity of the antennular chemoreceptors.

Single chemoreceptor cells from lateral filaments were identified electrophysiologically with a search stimulus (SS) composed of the following 15 compounds all at an applied concentration of 10^{-4} M: taurine, hydroxy-proline, glutamate, ammonium chloride, arginine, sucrose, ethanol, alanine, lysine, betaine, aspartate, glycine, leucine, glutamine and proline. The SS components were then tested individually at 10^{-4} M to determine the response spectrum for a particular cell. Applied concentrations were diluted by a factor of approximately 0.025 after introduction to the test chamber. Chemoreceptors especially sensitive to the single compounds hydroxy-proline, taurine, ammonium chloride, betaine and glutamate were found. These chemoreceptors had very restricted response spectra with little or mostly no response to the other test stimuli. Two additional chemoreceptors showed less specificity, one responded well to both proline and aspartate and the other responded to only the SS. Most of the chemoreceptors responded best to hydroxy-proline (59%); the next largest population was specialized for taurine (24%). The chemoreceptors which responded best to hydroxy-proline and taurine responded better when these stimuli were tested alone than when tested within the SS mixture. Thus, despite the restricted response spectra of these cells, the presence of the other test compounds reduced the stimulatory effectiveness of hydroxy-proline and taurine.

We suggest that narrowly tuned receptor cells may be useful to analyze the spatio-temporal properties of odor clouds which may contain directional information for orientation. Reduction of responses to individual compounds by the mixture is in marked contrast to behavioral data where mixtures are more effective stimulants than single compounds.

Supported by grants from the Whitehall Foundation and NSF (BNS 8210434).

295.24 SOMATOTOPIC MAPPING AND CHARACTERIZATION OF VISCERAL SENSORY NEURONS IN THE VAGAL LOBE OF THE CATFISH. Jagmeet S. Kanwal and John Caprio. Dept. of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803.

The vagal lobe (VL) is the primary center for visceral sensory (taste and tactile) input from the oro-pharyngeal region of teleosts. However, in Cyprinidae and Ictaluridae an additional structure, the facial lobe, receives visceral input from facial (VII) nerve branches innervating the extra-oral epithelium (barbels, lips and flank). The VL in these fishes is analogous to the caudal portion of the nucleus solitarius of mammals and receives afferents from the glossopharyngeal (IX) and vagal (X) nerves. Peripheral recordings indicated the presence of phasically responding taste and tactile units and tonically firing proprioceptive units in the IX and X nerves (Kanwal & Caprio, 1983). In the present study, chemical (amino acids) and mechanical stimulation of the oral cavity were employed to map the projections of the oro-pharyngeal sensory epithelium onto the VL in the channel catfish, Ictalurus punctatus. The three types of units characterized peripherally were also identified electrophysiologically within the VL. Taste activity was generally restricted to the superficial region (< 600 um deep) of the VL, while tonically firing units were present at greater depths (approx. 1 mm). The majority of the units observed in the VL were excited by mechanical stimulation, although a few inhibitory responses were also obtained. Vagal unit responses to mechanical stimulation of the oral epithelium revealed that the antero-posterior axis of each lateral half of the oral cavity is represented ipsilaterally in an anterior to posterior direction in the VLs. This is consistent with current neuroanatomical studies which indicate an antero-posterior pattern of projection of the peripheral (IX-X) neurons. Specific structures, such as the palatal organ, gill arches and tongue region, are represented in somewhat diffuse and overlapping domains. The anterior region of the VL contains cells which respond to ipsilateral as well as bilateral stimulation of the upper lip and proximal portion of the maxillary barbels. Thus, the VL preserves, in general, the spatial organization and electrophysiological nature of the visceral sensory input.

(Supported in part by NIH grant NS14819).

295.25 ULTRAVIOLET RADIATION EFFECTS ON OLFACTORY RECEPTOR RESPONSE. J.S. Kauer, J.O. Pretell* and K.A. Hamilton. Dept. of Neurosurgery, Tufts-N.E.M.C.; Dept. of Dermatology Harvard Med.Sch., Boston, MA.

Generation of an appropriate immune response requires both the recognition of antigen and the proper communication between cells of the immune system via specialized cell surface receptors. We have hypothesized that the surface membranes which confer chemical sensitivity on olfactory receptor neurons may be analogous to receptors on cells in the immune system. Ultraviolet (UV) irradiation of immune cells is known to interfere with the generation of an immune response (Bach, F.H. et al. Nature 239:273, 1976). Since UV has been shown to alter the turnover of several receptors on immune cells (Pretell, J.O. in Proc. of a Sci. Round Table on Effects of Ultraviolet Light on the Immune System ed. J.A. Parrish, Johnson and Johnson Press (in press), we examined whether UV might cause changes in the response of the olfactory mucosa as well. The EOG (electro-olfactogram) from the nasal epithelium of the salamander (Ambystoma tigrinum) was used to examine changes in response after UV irradiation of the exposed mucosa. Experiments were carried out on 12 animals in which the ventral nasal mucosa was opened and EOGs were recorded in response to controlled odor stimulation with amyl acetate and l-carvone. The mucosa was UV irradiated with a source having a major peak at 254nm (16000uW/cm2 at 8cm). EOG records were taken before, during, and after several short (1-2 min) pulses of UV separated by 7-15 min. Peak EOG amplitudes were plotted as percent of control obtained before UV pulsing. The results are as follows: EOGs from a single site on the mucosa after UV showed a decrement of response to 10-20% of control with 2-5 min total UV exposure. EOGs from two sites on the same mucosa, where one site was shielded from UV, showed a decrement at the unshielded site, but not at the shielded position. EOGs monitored for up to 36 hrs after UV showed a return to 60% or more of the control amplitude within 10-15 hrs. In summary, UV exposure of the salamander nasal mucosa causes a reversible decrement in the EOG. Toxic photoproducts, if formed, appear not to spread to unirradiated sites since EOGs from shielded portions of the same mucosa were unaffected. As an initial step toward determining the mechanism of the UV effects we are comparing the surface morphology using scanning EM of the shielded and unshielded portions of ventral mucosae. These experiments suggest that UV may reversibly change structures in the olfactory mucosa related to the generation of the EOG and therefore may be potentially useful in studying the initial components of the responses of the receptor cells to odor.

Supported by USPHS grant NS-17275 to JSK.

295.PO EFFECT OF THE DIURETIC BUMETANIDE ON THE SENSE OF TASTE. S. S. Schiffman and E. M. Lockhead*. Dept. of Psychiatry, Duke Medical Center, Durham, NC 27710.

The potent new diuretic drug bumetanide, 3-N-butylamino-4-phenoxy-5-sulfamylbenzoic acid, is known to increase secretion of Na^+, Cl^-, and K^+ in the human kidney. Recent evidence suggests that bumetanide is an inhibitor of $Na^+ + K^+ + 2Cl^-$ co-transport. In order to determine whether bumetanide blocks specific ion channels involved in taste, this diuretic was applied to the human tongue. Two pieces of filter paper cut in the shape of half-tongues were placed on the dorsal tongue surface for a total of 5 minutes. One filter paper was impregnated with 10^{-4}M bumetanide. The other was soaked in deionized water. Circles of filter paper with a diameter of 1/2 inch impregnated with taste stimuli were placed at symmetrical areas on the two sides of the tongue. The side of the tongue adapted to bumetanide was presented salts (.2, .4, and .6 M NaCl, .3 M KCl, .2 M choline Cl), sweeteners (.8 M sucrose, .0011 M stevioside), amino acids (.2 M L- and D-alanine), acids (.01 M citric acid, .01 M HCl), and bitter compounds (.002 M QHCl, 1.5 M urea). The concentrations required to match those on the bumetanide side were determined. The results indicate that bumetanide reduces the intensity of NaCl, KCl, and all sweet-tasting compounds. It also reduced the intensity of QHCl.

SYNAPTIC STRUCTURE AND FUNCTION I

296.1 MORPHOLOGICAL EVIDENCE FOR BIDIRECTIONAL CHEMICAL SYNAPSES. Gary McCarragher* and Ronald Chase. Dept. of Biology, McGill University, Montreal, Quebec, H3A 1B1.

Recent observations in diverse nervous tissues have disrupted the conventional concept of the functional polarity of axon and dendrite. We now report findings in the tentacle ganglion of the terrestrial snail Achatina fulica which further erode this concept. The ganglion consists of a thin rind of perikarya surrounding a finely structured, dense neuropil. Two ganglia were fixed with 1% gluteraldehyde in 0.05 M phosphate buffer (pH 7.4), and postfixed with 1% O_sO_4. Thin sections (70-100 nm) were stained with uranyl acetate and lead citrate. Grids were scanned systematically and all candidate synapses were photographed. A total of 33 active zones were subsequently identified as satisfying the following criteria: sharply defined and rigidly opposed membranes, membrane densification, vesicle accumulation, and the presence of cytoplasmic dense projections associated with at least one of the opposed membranes. Many of these synapses have a symmetrical appearance. In order to quantify this impression, the density of vesicles within 20 nm of the active zone was measured, on both sides of the synapse. For most synapses, the ratio of vesicle accumulations high side: low side was infinite. However, in 10 cases the ratio high side: low side was <2.5. For synapses in which the ratio of vesicle accumulations was >2.5, dense projections were rarely found on both sides of the synapses (1 of 24). Conversely, when the ratio was <2.5, dense projections were present on both sides of the synapse in all but one case. Thus, the convergence of symmetrical vesicle accumulations (ratio <2.5) and symmetrical dense projections resulted in the designation of 9 bidirectional synapses (27% of total sample). The vesicle accumulation at the low side of the bidirectional synapses (mean, 660 vesicles/μm^2) is significantly greater ($p < .01$) than at systematically sampled non-active regions of the same membrane (mean, 40 vesicles/μm^2) but not significantly different from the accumulation at unidirectional synapses (mean, 444 vesicles/μm^2). The accumulation at the high side of bidirectional synapses (mean, 890 vesicles/μm^2) is greater than at unidirectional synapses ($p < .01$). The unidirectional or bidirectional character of a synapse was maintained throughout serial sections. Moreover, serial sections show that plane of section artifacts cannot account for the results. These observations suggest that the concept of reciprocal interaction between neurons be extended to include the possibility of bidirectional communication at a single locus of chemical transmission.

296.2 MORPHOLOGY AND PHARMACOLOGY OF PRESYNAPTIC INHIBITION MEDIATED BY PUTATIVE HISTAMINERGIC NEURONS IN APLYSIA. C.H. Bailey*, M. Chen, R. Kretz* and E. Shapiro. Center for Neurobiology & Behavior, Depts. Anatomy and Cell Biology, Neurology and Psychiatry, Columbia University, P & S and the N.Y.S. Psychiatric Institute, New York, N. Y. 10032.

The output of neuron L10 to identified RB and left upper quadrant follower cells is presynaptically inhibited by a group of identified neurons, the L32cells (Byrne, 1980). Utilizing voltage-clamp techniques, Kretz et al. (in preparation) have recently examined the conductance changes underlying this presynaptic inhibitory interaction. Their studies suggest that one component of L32's inhibitory effect may result from a transmitter-mediated reduction in the Ca^{++} current at L10's terminals.

We have examined the fine structure and synaptic organization of the L32 cells following intrasomatic injection of horseradish peroxidase (HRP). L32's axonal tree consists of small (1-3 μm) varicose expansions interconnected by thin (0.1-0.5 μm) microtubule-filled neurites. Elongated sacs and flattened cisternae are occasionally found in both the intervaricose segments and synaptic terminals, often occupying a subsurface configuration. Each varicosity contains two general classes of vesicles. The most frequent vesicle profile is relatively electron-lucent with a mean long diameter of 67 nm $\pm$ 18 nm S.D., N=3024. A frequency distribution histogram suggests the lucent population is composed of two subclasses with peaks at 60 nm and 85 nm. The second general class of vesicles are larger (mean long diameter 98 nm $\pm$ 17 nm S.D., N=682) and contain an electron-dense core that typically fills each vesicle. The size and morphology of the vesicle population in L32's terminals is remarkably similar to that described at the synapses of the identified histaminergic neuron C_2 in Aplysia (Bailey et al., 1982; Schwartz et al., 1983).

This finding suggested presynaptic inhibition mediated by L32 may be due to histamine. We found that ionophoresis of histamine onto L10's soma produced a slow hyperpolarization of L10, similar to that caused by L32 stimulation. Both the L10 histamine response, and the L32 PSP onto L10 could be specifically and reversibly blocked by cimetidine (10^{-4}M), a histamine antagonist. Bath application of histamine (10^{-4}M) specifically inhibited L10's transmitter output onto follower cells, mimicking presynaptic inhibition. Preliminary voltage-clamp experiments revealed that histamine increased a TEA^+-sensitive K^+ conductance and reduced a voltage-dependent Ca^{++} conductance in L10, again similar to the physiological effect of L32 stimulation. These results are consistent with the possibility that the L32 cells, identified interneurons mediating presynaptic inhibition in Aplysia, are histaminergic. We are currently exploring this possibility biochemically.

296.3 SYNAPTIC VESICLES CONTAINING SUBSTANCE P BIND A MONOCLONAL ANTIBODY SPECIFIC FOR SYNAPTIC VESICLES. E. Floor and S.E. Leeman. Univ. Massachusetts Medical School, Worcester, MA 01605.

Synaptic vesicles that contain substance P (SP) have been purified 75-fold from rat brain (Floor et al. (1982) Neuroscience, 7, 1647). Monoclonal antibodies that recognize a 65kd protein on the surface of synaptic vesicles in a wide range of neuronal types have been isolated and characterized (Matthew et al. (1981) J. Cell Biol. 91, 257). The antigen recognized by these antibodies is shown here by immunoprecipitation to be present on substance P-containing vesicles from brain. Vesicles containing SP were purified by chromatography on controlled pore glass (CPG) beads as described previously. After overnight incubation at 4°C with antibody 48 (from W.D. Matthew), vesicles were precipitated by centrifugation at 7,500g after an additional incubation with second antibody-coated beads (rabbit anti-mouse Immunobeads, BioRad). In this series of experiments the amounts of substance P in the large (85-110nm) and small (30-85nm) vesicle fractions from the CPG column were similar (cf. Floor et al., 1982), so that particles of both size ranges could be studied. In the absence of either first or second antibody, precipitation of SP was minimal. Immunoprecipitation of substance P from both large and small vesicle fractions increased roughly in parallel and in a saturable manner with antibody 48 concentration. At the highest concentration used to date, more than 60% of the total vesicular SP in either large or small vesicle fractions was precipitated. Total vesicular SP was determined as the amount of SP sedimented after 30 min at 100,000g and was about 40% of total SP in the preparation.

These results strongly confirm that most of the large SP-containing vesicles in the preparation are indeed synaptic vesicles and show that these peptidergic vesicles from brain share a surface antigen in common with many other neurotransmitter vesicle types. They also indicate that substance P is associated with small synaptic vesicles; whether SP is inside or outside the vesicles is not known at the time of writing.

Supported by an NIH Biomedical Research Support Grant (E.F.) and NIH Grant AM29876 (S.E.L.).

296.4 EFFECTS OF CHRONIC ADMINISTRATION OF PYRIDOSTIGMINE AT THE NEUROMUSCULAR JUNCTION. C.K. Meshul, S.S. Deshpande, A.F. Boyne and E.X. Albuquerque. Department of Pharmacology and Experimental Therapeutics, Univ. of Maryland Medical School, Baltimore, Md., 21201.

We have found that a single subcutaneous injection of sarin (80 µg/kg), an irreversible anticholinesterase (anti-ChE), produced ultrastructural damage localized to the endplate (EP) region of the soleus but not the extensor digitorum longus muscles in the rat (Fed. Proc., 42, 655, 1983). We are evaluating the working hypothesis that this differential damage is caused by excessive ion accumulations within the EP due to prolonged agonist activity of acetylcholine (ACh) released into the synaptic cleft. Thus, it was of importance to investigate if this effect of sarin could also be seen with a reversible and clinically used anti-ChE, such as pyridostigmine (Pyr). A dose of 9-12 mg/kg/day of Pyr was given subcutaneously to female Wistar rats (200g) in 3 divided doses for 1 to 7 days. The resting membrane potential and spontaneous miniature endplate potentials (mepps) were recorded at the EP region of surface fibers of the fast extensor digitorum longus and the slow soleus muscles at day 1, 4, and 7. These muscles were then prepared for electron microscopy. After one day, the soleus EPs showed prolonged mepps with a 50% amplitude reduction. Ultrastructurally, these EPs showed a loss of postjunctional folds and an accumulation of dark and lucent membrane-bound vesicles/vacuoles and a pronounced loss of muscle sarcoplasmic reticulum membrane. By day 4, complete separation of the intact nerve terminal from the underlying muscle was seen. These changes persisted to day 7. As seen with sarin, the extensor muscle was much less affected by this in vivo drug treatment. The observations are consistent with the hypothesis that drugs which prolong agonist activity at nicotinic receptors in vivo will eventually cause excess ion accumulations in the muscle cytoplasm of the EP. The vacuoles which are developed in this situation may draw membrane from the sarcoplasmic reticulum and may represent an attempt to sequester the incoming ions. These vacuoles appear to mediate detachment of the apparently normal nerve terminal from the muscle surface. This novel finding suggests that Pyr, at the concentrations used in this study, can cause damage at the EP. A direct action of pyridostigmine on the ACh receptor may exacerbate effects due to inhibition of the AChE. (Fed. Proc., 42, 991, 1983). (Supported by U.S. Army Medical Research and Development Command Contract No. DAMD 17-81-C-1279.)

296.5 SYNAPTIC TRANSMISSION BLOCKADE BY THE IONOPHORE A23187. PHYSIOLOGICAL AND ULTRASTRUCTURAL STUDIES. F.T. Llados, J. Ross-Canada*, R.P. Becker* and G.D. Pappas. Dept. Anatomy, Univ. Illinois, Chicago, IL 60612.

The effect of the ionophore A23187 on spontaneous miniature endplate potentials (MEPPs) at the neuromuscular junction has been well documented. Briefly, MEPP frequency has been reported to transiently increase in a dose-dependent manner within minutes after the application of the ionophore to the neuromuscular preparation in vitro. Changes in MEPP amplitude (due to presynaptic action) have been difficult to assess due to the depolarizing effect of the drug on the muscle fiber membrane.

The present study was aimed to determine the ultrastructural effects of the ionophore A23187 at the frog neuromuscular junction. Sartorius nerve-muscle preparations were dissected from Rana pipiens and placed in an incubating bath at room temperature. Both muscles were dissected, one serving as control for the other. MEPPs were recorded intracellularly with conventional 3M KCl glass microelectrodes. After recording MEPPs for 10 to 15 minutes the ionophore was added to the bath to achieve a final concentration of 10 to 30 µ M. After a variable period of 5 to 15 minutes, depending on the dose, MEPP frequency started to gradually increase until single events could not be distinguished (200/sec or higher). At this point the preparation was fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4 and prepared for examination of conventional plastic sections. No obvious morphological differences were found between experimental and control endings with regard to synaptic vesicle number or distribution of vesicles within the terminal. However, after 45 minutes of exposure to the ionophore (when MEPP frequency had declined almost to zero) most of the nerve endings examined appear devoid of synaptic vesicles and other organelles while maintaining intact the plasma membrane. The latter was also confirmed in resinless sections from which the embedding medium, polyethylene glycol, had been removed.

It is suggested that the apparent depletion of vesicles from the terminal induced by the ionophore is a consequence of irreversible exocytosis of the synaptic vesicle population. The condition produced by the ionophore resembles that produced by black widow spider venom and lanthanum ions.

296.6 ULTRASTRUCTURAL CORRELATES OF DIFFERENCES IN SYNAPTIC EFFECTIVENESS AT FROG NEUROMUSCULAR JUNCTIONS. A.D. Grinnell, A.A. Herrera, and B. Wolowske* (SPON: B. Nudell). Jerry Lewis Neuromuscular Research Center, UCLA, Los Angeles CA 90024. AAH at Dept. Biological Sciences, Univ. of Southern California, Los Angeles CA 90089.

There are sharp differences in synaptic effectiveness at neuromuscular junctions in the cutaneous pectoris (CP) and sartorius muscles of the frog Rana pipiens, due to differences in transmitter release (J. Physiol. 307, 301). The light microscope, however, reveals no morphological differences. To see whether such differences in release have an ultrastructural basis, we used the electron microscope to compare junctions in a CP and a sartorius muscle that showed a typical difference in safety margin, i.e., CP junctions were substantially stronger. In the CP 116 nerve terminal cross sections from 62 randomly selected endplates were examined while in the sartorius 94 terminal cross sections from 48 endplates were sampled. No more than one section was taken through a given endplate. Sections passing through an active zone (AZ) were analyzed separately from those through regions between AZs. Mean nerve terminal diameter, measured parallel to the muscle fiber surface, did not differ significantly between the CP and sartorius either at AZs (CP 1.99 µm, sart. 1.79 µm) or between AZs (CP 1.31 µm, sart. 1.27 µm). However, as these numbers show, both types of terminals are varicose along their length, with AZ regions wider than between AZs. Significant differences were seen in the mean width of presynaptic membrane in close apposition to postsynaptic membrane, both at AZs (CP 2.20 µm, sart. 1.65 µm) and between AZs (CP 1.26 µm, sart. 0.71 µm). This difference is of particular importance in explaining the disparity in transmitter release since apposition width is probably roughly equivalent to AZ length. The greater apposition width of CP terminals at AZs is at least partly explained by the significantly lower incidence of interposition of Schwann cell processes between pre- and postsynaptic membranes also found there: only 5% of the CP terminal membrane within 0.2 µm of the muscle membrane was obstructed by Schwann cell processes while 14% of the terminal membranes in the sartorius were so obstructed. Between AZs there were large differences in Schwann cell interposition: 16% for the CP and 51% for the sartorius, using the index of interposition described above. These results confirm earlier findings on ultrastructural correlates of long-term enhanced release at identified motor nerve terminals (Soc. Neurosci. Abstr. 8, 492) and emphasize the importance of the AZ in the long term regulation of transmitter release levels.

Supported by USPHS grants NS06232 (ADG) and NS18186 (AAH).

297.7 Synaptic vesicle number and size correlated with amplitude of post synaptic potentials. D.C. Miller, Dept. of Physiology & Biophysics U.Miami Sch. Med., Miami,FL 33101, and Dept. of Pathology (Neuropathology), Mass. General Hospital, Boston, MA 02114.

The vesicle hypothesis for transmitter release proposes that each quantum of release arises from the exocytotic release of the contents of one synaptic vesicle. At the neuromuscular junction there is much evidence to support the hypothesis but no direct relationship has been demonstrated between vesicle size and end-plate potential amplitude. Furthermore it has been asserted that fixation in solutions containing high concentrations of Mg^{2+} reveals "extra" vesicles not seen in conventional fixatives and that the "true" number of vesicles exceeds the number of available quanta (Birks, RI,J.Physiol. 216:26P-,1971, and J.Neurocytol. 3: 133-,1974;McKinlay,RG, & Usherwood,PNR,J.Ultrastruct.Res.62:83-, 1978). This Mg effect was attributed to Mg-induced blockade of fixative-related depolarization-induced exocytosis. To test this hypothesis frog cutaneous pectoris neuromuscular junctions were incubated and fixed in solutions containing either 2 mM Ca^{2+} alone or with 15 mM Mg^{2+} or 5 mM Co^{2+} added. High Mg^{2+} fixation yielded terminals with some areas packed by vesicles similar to those previously reported; other areas, however, appeared empty. Fixation in the presence of 5 mM Co^{2+} did not produce this effect. These results favor the hypothesis that raising the Mg^{2+} concentration non-specifically aggregates synaptic vesicles, just as it does in synaptosomes (Gray,EG, Jones,DH, & Barron,J, J.Neurocytol. 8:675-,1979)and with liposomes (Miller,DC, & Dahl,GP, Biochim. Biophys. Acta 689:165-,1982).The results do not suggest the presence of extra vesicles, and do not support the hypothesis that high Mg^{2+} concentrations in fixatives inhibit loss of vesicles, since Co^{2+} is a more potent blocker of release at equimolar concentrations.

Histograms of the diameters and calculated volumes of vesicles in the nerve terminals were compared to previously measured histograms of miniature endplate potential amplitudes (mepps). Diameter histograms were fit well by Gaussian curves, and produced volume histograms that were clearly non-Gaussian with a large proportion of large volumes. Stereologic corrections did not alter this result. No group of small diameter/volume vesicles corresponding to the small ("subminiature") mepps was seen. Since mepp amplitude histograms are fit well by Gaussian curves (Magleby KL & Miller DC, J.Physiol. 311:267-,1981) these data show that there is no direct relationship between vesicle volumes and mepp amplitudes. If the vesicle hypothesis is correct, either vesicles are filled to variable concentrations of transmitter or release varying proportions of their contents upon exocytosis.

296.8 PHYSIOLOGICAL DIFFERENCES BETWEEN STRONG AND WEAK FROG NEUROMUSCULAR JUNCTIONS. Peter A. Pawson and Alan D. Grinnell . Jerry Lewis Neuromuscular Research Center, UCLA, Los Angeles, CA., 90024.

Neuromuscular junctions (NMJs) within the frog sartorius muscle differ over a 10-fold range in transmitter release per unit length (Grinnell & Herrera,J.Physiol.1980). We are attempting to define the physiological mechanisms which underlie these differences in transmitter release. Strong NMJs show an increase both in evoked (EPP) and spontaneous quantal release(MEPPS). These increases suggest that the intraterminal active [Ca^{2+}] may be regulated at a higher concentration than normal. To test this hypothesis, we have studied the properties of posttetanic potentiation (PTP) in strong vs. weak junctions, since the time constant of the decay of potentiation (τ_p) gives an indication of the kinetics of Ca^{2+} metabolism in junctions of varying synaptic strengths.

We have adopted the experimental design of Lev-Tov & Rahamimoff (J.Physiol.1980). Following the assessment of synaptic strength in a low Ca^{2+} Ringer, identified junctions were restudied in a Ringer solution (0.1mM Ca^{2+}, 5mM Mg^{2+}) in which little if any evoked release occurs, even during a tetanus (50 Hz,40 sec.). All junctions show a progressive increase in MEPP frequency during a tetanus. Thereafter, MEPP frequency typically decays back to the baseline level in a double exponential manner, with the slowest exponent representing τ_p. We have studied 11 identified endplates from 3 muscles, representing a 9-fold range of synaptic strengths. Within this sample there is a strong positive correlation (r=0.74) between increasing synaptic strength (range: 0.14-1.33 quanta/100um) and increasing τ_p (range: 21.8-240.0 sec.). The consistency of the findings indicates that an increased potential for PTP represents an heretofore unrecognized attribute of strong NMJs. We interpret these results as indicating that strong NMJs have 1) a reduced Ca^{2+}-buffering capacity and/or that 2) they have a proportionately greater Ca^{2+}-influx during the tetanus, thereby leading to a saturation of the Ca^{2+} buffering system. We are presently conducting similar experiments in 0 Ca^{2+}, EGTA Ringers to separate the roles of Na^+ and Ca^{2+} in elaborating the physiological differences in τ_p. We will also study the effects of a reduced Ca^{2+} influx into strong junctions on the long τ_p of these terminals.

(Supported by a MDA fellowship to P.A.P. and research grants from the MDA and USPHS).

296.9 ENLARGED SYNAPTIC VESICLES ASSOCIATED WITH INCREASED QUANTAL SIZE IN A DROSOPHILA MUTANT. J.H. Koenig and K. Ikeda. Divison of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

The temperature sensitive mutant, shibire, of Drosophila melanogaster, demonstrates a reversible block in synaptic transmission at 29°C. It has been shown that with moderate activity, a reduction in the amplitude of the excitatory junction potential (ejp) and a reduction in the frequency of spontaneously released miniature excitatory junction potentials (mejp's) occurs at 29°C in the neuromuscular junctions of the dorsal longitudinal flight muscle (DLM) of this mutant (Koenig et al., 1983). It has also been shown that at 29°C, the DLM synapses contain almost no vesicles, but instead contain some large cisterna-like structures, as well as pit-like structures on the synaptic membrane (Saito and Ikeda, 1979). It has been suggested that the reduction in ejp amplitude and mejp frequency is due to vesicle depletion, which is caused by a block in the recycling of synaptic vesicles while exocytosis (transmitter release) proceeds uninhibited. The block in recycling appears to be at the step where pits pinch off from the membrane to form vesicles.

The temperature induced changes mentioned above are reversible if the temperature is returned to 19°C. The process of recovery was observed at the DLM synapses, and it was noted that the large, cisterna-like structures disappear, and instead vesicle-like structures, many of which are larger than the typical vesicles of this synapse, are observed. At a time when these unusually large vesicle-like structures are observed, it was also observed physiologically that the mejp's are larger in amplitude than typical mejps which are observed in wild-type flies or shi flies which were not heated. Thus, it was observed that when the diameter of the vesicles is increased, the amplitude of the mejp's is also increased. (Supported by USPHS Grant NIH 18856).

296.10 MEMBRANE SPECIALIZATIONS AT INHIBITORY AND EXCITATORY SYNAPSES ON THE GOLDFISH MAUTHNER CELL. R. Tuttle*, S. Masuko*, and Y. Nakajima. Dept. of Biological Sciences, Purdue University, West Lafayette, IN 47907.

The inhibitory and excitatory synapses on the distal part of the lateral dendrite of the goldfish Mauthner cell have been studied using the double replica freeze fracture method. Only two types of synaptic endings terminate on the distal lateral dendrite: Small-vesicle boutons (SVBs), and large myelinated club endings (LMCEs) (1). The SVBs form chemical inhibitory synaptic contacts at which glycine could be the transmitter (2). The LMCE forms a mixed synapse having both excitatory electrical and chemical synaptic junctions (1,3,4,5).

The P face of the postsynaptic membrane at a SVB is characterized by a high density of small to medium sized intramembrane particles (IMPs), and usually one or more irregularly shaped aggregates. In contrast, the postsynaptic E face is characterized by an aggregate which is composed of large IMPs, is generally round or ovular in shape, and is of variable size. The presynaptic membrane at a SVB contains many E face protuberances and complementary P face depressions which presumably represent sites of synaptic vesicle interaction with membrane. These sites appear to be distributed irregularly over much of the presynaptic membrane.

The postsynaptic membrane at a LMCE has two types of IMP aggregates. One of them consists of numerous gap junction IMPs. The second aggregate type is in the postsynaptic E face and consists of large IMPs. These aggregates are large at the periphery of the LMCE. The presynaptic P face contains active zone-like structures consisting of large IMPs and depressions corresponding to E face protuberances. These presumptive active zones are most noticeably localized along the periphery of the LMCE and appear to correspond to the postsynaptic E face aggregates. This membrane structure is characteristic of excitatory chemical synaptic junctions previously described in the central nervous system.

In conclusion, our study has clearly demonstrated the presence of membrane specialization in both the P and E face of an inhibitory synapse. However, whether IMPs present in either the P or E face aggregates represent receptor molecules or not remains unclear. The structures which were seen at the LMCE correlate well with previous evidence from thin-section and physiological studies (1,3,4,5). (Supported by NSF Grant BNS 8106673).

1. Nakajima, Y. (1974) J. Comp. Neurol., 150:375-402.
2. Werman, R. (1980) In Neurotransmitters & Their Receptors. eds. Littauer et al. John Wiley & Sons, New York, pp. 393-404.
3. Brightman, M.W. & Reese, T.S. (1969) J. Cell Biol. 40:648-677.
4. Furshpan, E.J. (1964) Science 144:878-880.
5. Faber et al. (1980) Neuroscience 5:433-440.

296.11 ULTRASTRUCTURE OF A PHASE COMPARISON CIRCUIT IN THE MIDBRAIN OF A WEAKLY ELECTRIC FISH. C. E. Carr, B. Taylor and L. Maler. UCSD, A-002,La Jolla, CA, 92093 and Dept. Anatomy, Univ.of Ottawa, Ottawa, Ontario K1H 8M5, Canada.

The gymnotoid fish Eigenmannia produces a high frequency electric organ discharge (EOD) which it uses for electrolocation and social interactions. These fish possess a Jamming Avoidance Response (JAR) whereby they raise or lower the frequency of their EOD to avoid being jammed by a neighbouring fish at a similar frequency. This response requires that the fish be able to evaluate the simultaneous changes in amplitude and phase which occur when its EOD is contaminated with that of a neighbour's, and then that it produce an appropriate shift in its EOD frequency.

These changes in amplitude and phase are processed in the midbrain torus. Afferents from the medulla project somatotopically to different laminae. Phase information projects only to lamina 6. We are using intracellular recording and electron microscopic analysis of HRP filled neurons to identify the elements of the phase comparison circuit.

Phase coder afferents from the contralateral medulla terminate as dense baskets of terminals on and around the somas of the giant (40μ) interneurons characteristic of this lamina. The afferents form large club-shaped endings which terminate on the soma of the giant cell. They contain a dense matrix of mitochondria,neurofilaments and microtubules, as well as large numbers of vesicles, coated vesicles and dense core vesicles, none of which appear to be associated with the presynaptic membrane. The afferents make extensive gap junction contacts with the giant cells. The gap junctions are asymmetric, with a dense region made up of globular subunits on the postsynaptic side. That part of the giant cell soma which is not covered with afferent terminals is wrapped in glial processes.

The giant cell is electron dense, due to the presence of large numbers of intracellular organelles, particularly mitochondria,the Golgi apparatus and rough endoplasmic reticulum. The cells are all unipolar with 2-3 large(10μ) axons which project to distant portions of lamina 6, and a number of finer processes which arborize close to the soma.

Lamina 6 contains two other cell types. These cells are small (5-10u) uni- or bipolar neurons scattered throughout the lamina and sometimes grouped in clusters of 2-3 cells. Type I is electron dense with a large number of mitochondria and rough endoplasmic reticulum. Type II has fewer organelles and a larger nucleus. Golgi studies suggest that both types I and II project out of lamina 6 to the amplitude coding laminae above and below lamina 6.

Supported by NSF Grant BNS82-05454 to W. Heiligenberg.

296.12 ORIGIN OF THE ACETYLCHOLINESTERASE MOLECULES FOCALIZED AT THE NEUROMUSCULAR CONTACTS IN VITRO. J. KOENIG[1,2], S. DE LA PORTE*[2], D. MARSH*[3], J. GRASSI*[4], F. VALLETTE*[3] and M. VIGNY*[3]. 1: Inserm U.153 - 17 rue du Fer-à-Moulin 75005 PARIS, 2 : Université P.et M. CURIE PARIS. 3 : Laboratoire de Neurobiologie E.N.S. Paris, 4 : Departement Biologie LERI CEN Saclay.

It has been shown by cytochemical method that acetylcholinestérase molecules (AChE) are focalized at the neuromuscular synapses in vivo as well as in vitro. This enzyme exhibits 2 classes of molecules : the asymmetric (A) and the globular forms (G). It has been shown that AChE is in part associated with the basal lamina located between the nerve ending and the muscle. The A_{12} asymmetric form has aroused much interest because in rat muscle it is specifically localized at the end-plate and its presence is controlled by innervation. Furthermore the neuronal AChE is transported by the axonal flow in particular the entirety of the minor A_{12} is transported by the fast axonal flow. Thus a partially neuronal origin of the enzyme concentrated at the end-plate cannot be excluded.

In order to investigate this problem, we employed heterologues synapses formed in vitro between muscles and neurons of the rat and the chick and analysed the enzymic forms localized at neuromuscular contacts. AChE was visualized using fluorescently labelled monospecific antibodies raised either against the rat AChE or against the chick AChE and showing no cross-reactivity. Acetylcholine receptor clusters were identified by labelling with rhodamine labelled α bungarotoxin. It is noteworthy that in muscle cells alone AChE patches were absent but were induced at the neuromuscular contacts by the presence of nerve cells. The sedimentation coefficient of the A_{12} form of chick and rat enzymes are quite distinct (20S and 16S) and can be resolved by centrifugation analysis. Thus, in the heterologues co-cultures the nature of the asymetric forms synthesized in the presence or in the absence of nerve cells can be easily demonstrated.

Our data are consistant with a totally or largely predominant muscular origin of the enzyme associated with neuromuscular contacts in vitro.For example, in the rat myotube-chicken neuron system we were able to detect the patches of enzyme only by using the anti-rat AChE antibodies and the asymmetric forms were only represented by the 16S and the 12S forms corresponding to the rat enzyme.

296.13 SYNAPTIC VESICLES AND THE SYNAPTIC CLEFT CONTAIN AN IDENTICAL PROTEOGLYCAN. S.S. Carlson, K.M. Buckley, P. Caroni* & R.B. Kelly. Dept of Biochem & Biophys., University of California, San Francisco, Ca 94143

Although exocytosis involves the fusion of a secretory vesicle membrane with a plasma membrane, the protein composition of the two membranes is thought to be different. We have tested this hypothesis using nerve terminals from the electric organ of marine rays. Antibodies that bind to the external surface of the nerve terminal can be identified because they allow selective immunoadsorption of synaptosomal contents (Mijanich et al. (1982) J. Cell Biol. 94, 88). We have used immunoadsorption to screen a library of monoclonal antibodies raised to electric organ nerve terminals by Drs. Kushner and Reichardt (UCSF). Of the 16 positives, 15 show no detectable binding to synaptic vesicles. The 16th, mAb70, binds strongly to synaptic vesicle membranes. The antigen recognized by mAb70 is a proteoglycan-like molecule associated with the luminal surface of the vesicle membrane. Since it is the major unique antigen in synaptic vesicles, we had already purified the proteoglycan-like molecule. It contains a heparin-like glycosaminoglycan, has approximately equimolar amounts of uronic acid and N-acetylglucosamine and a total molecular weight of about 200 kd, about 30% of which is carbohydrate. The location of the antigen on the outside of nerve terminals was determined by immunoelectron microscopy using an HRP-labeled second antibody procedure. Binding of mAb70 was restricted to the synaptic cleft. An attractive interpretation of this result is that since synaptic vesicles only insert in the membrane at the synaptic cleft, they are vehicles for the insertion of cleft-specific extracellular matrix.

296.14 PHOSPHORYLATION OF SYNAPTIC GLYCOPROTEINS. K. Kryzwicka*, N. Bissoon* and J. W. Gurd* (SPON: I. R. Brown). Dept. of Biochemistry, Scarborough College, University of Toronto, West Hill, Ont., Canada M1C 1A4.

Postsynaptic structures of synapses of the central nervous system are associated with a unique complement of high molecular weight glycoproteins. We have previously demonstrated the phosphorylation of these glycoproteins following incubation of isolated synaptic junctions (SJs) with γ-AT^{32}P (Brain Res. in press). We have now further characterized the phosphorylation of these synaptic specific components by investigating the incorporation of ^{32}P into glycoproteins following in vitro incubation of synaptic membranes (SMs) with γ-AT^{32}P and the in vivo administration of inorganic ^{32}P. Synaptic membranes were incubated with AT^{32}P and glycoproteins isolated by con A affinity chromatography. Maximum incorporation of ^{32}P into glycoproteins was attained within 15 to 30 sec following which radioactivity decreased to low levels. This short time course of incorporation contrasts to results obtained with SJs in which labelling continued to increase for 5 to 10 min and indicates the action of protein phosphatase(s) on synaptic glycoproteins. Synaptic membranes which had been incubated with γ-AT^{32}P were extracted with Triton X-100 to obtain SJs and con A$^+$ glycoproteins were isolated by affinity chromatography. The previously identified SJ glycoproteins with apparent molecular weights of 110K, 130K and 180K incorporated ^{32}P with the latter species being the most highly labelled. Inorganic ^{32}P was administered by intracerebral injection and SJs isolated 30 to 120 minutes later. Several SJ proteins were labelled with ^{32}P. Isolation of con A$^+$ glycoproteins followed by gel electrophoresis and radioautography demonstrated the incorporation of ^{32}P into GP 180. These results demonstrate that postsynaptic glycoproteins, and in particular GP 180, are substrates for intrinsic synaptic protein kinase(s) and phosphatase(s).

Supported by grants to JWG from the Canadian Medical Research Council and National Science and Engineering Research Council.

296.15 STUDIES ON THE ORIENTATION OF SYNAPTIC PLASMA MEMBRANE PROTEINS USING A NON-SPECIFIC PHOTOACTIVATED LABELING REAGENT. J.C.Schaeffer, Dept. of Chemistry, California State U., Northridge, CA 91330 and E.J. Gregory*, Dept. of Chemistry, University of Missouri, Kansas City, MO 64110.

The orientations of proteins associated with synaptic plasma membranes (spm) from rat brain were investigated using a non-specific, non-permeable, photoactivated labeling reagent: tritiated 2-(4-azido-2-nitroanilino)ethyltrimethylammonium iodide. Intact synaptosomes associated with the P_2 brain fraction, intact synaptosomes purified on a Ficoll gradient, and spm isolated from lysed synaptosomes were labeled. After the labeled spm were isolated, thoroughly washed and solubilized, the molecular weights and isoelectric points of their tritiated protein components were determined by SDS gel electrophoresis or isoelectric focusing, respectively.

When the spm of intact synaptosomes associated with the P_2 brain fraction were photolabeled, membrane proteins in the MW ranges 99 to 169Kd, 45 to 75Kd, and 29 to 42Kd were prominently tritiated with the relative intensity of 1:1.5:1.8, respectively. Labeling of the spm of purified intact synaptosomes produced a different protein labeling pattern. A major feature of which was a marked decrease in tritium incorporation in the 29 to 42Kd region. In addition to the spm proteins labeled in intact synaptosomes, a major new peak of tritium incorporation with an apparent MW of 19Kd was observed when spm from lysed synaptosomes were labeled.

For all three preparations that were labeled, isoelectric focusing revealed major tritium incorporation into spm proteins whose isoelectric points were in two broad ranges centered at pH 5.25 and pH 9. However, the relative tritium content of the pH 9 region was very dependent on the preparation that was labeled: intact synaptosomes in P_2 brain fraction < purified intact synaptosomes < spm from lysed synaptosomes.

These data suggest that proteins with a broad range of molecular weights are exposed on the external surface of spm, that certain spm proteins may be lost or rearranged during synaptosome purification, and that a 19Kd basic protein may be associated with the internal spm surface. Supported in part by DA-03319.

296.16 STUDIES ON THE SUBCELLULAR AND REGIONAL DISTRIBUTION OF CALMODULIN-DEPENDENT PROTEIN KINASE II IN RAT BRAIN. T. L. McGuinness*, P. T. Kelly†, C. C. Ouimet and P. Greengard (SPON: R. A. Nichols). Dept. Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510, and †Div. Biology, Kansas State Univ., Manhattan, KS 66506.

A calmodulin (CaM)-dependent protein kinase, CaM kinase II, has been purified from rat brain on the basis of its ability to phosphorylate Synapsin I, a synaptic vesicle-associated neuronal protein. The purified kinase contains autophosphorylatable subunits of 50 kdaltons (kD) and 60 kD, both of which bind CaM. The subcellular and regional distribution of these peptides in rat brain have been examined.

Postsynaptic densities (PSDs) contain a CaM-dependent kinase that phosphorylates endogenous 50 kD (the major PSD protein) and 60 kD peptides (Grab et al., J.C.B., 89, 440, 1981). Both of these phosphopeptides appear to be CaM-binding proteins. The 50 kD and 60 kD phosphoproteins in PSD enriched fractions are indistinguishable by a variety of criteria from those in CaM kinase II. These criteria include: 1-dimensional ^{32}P-labeled proteolytic peptide maps, 2-dimensional ^{125}I-labeled tryptic/chymotryptic peptide fingerprints, and cross-reactivity on Western blots with a variety of polyclonal and monoclonal Abs. These results suggest that CaM kinase II may be responsible for mediating some Ca^{2+}-dependent effects at the postsynaptic element. The enzyme, however, is not localized exclusively in PSDs, since approximately 50% of the kinase II present in whole brain homogenates is cytosolic and much of the remaining particulate enzyme can be easily extracted (Kennedy et al., J. Neurosci., 3, 818, 1983).

Immunocytochemistry, using a monoclonal Ab that recognizes the 50 kD and 60 kD peptides, reveals immunoreactivity throughout the brain, with regional variation in staining intensity. Areas of high immunoreactivity include: dentate gyrus, amygdala, septum, indusium griseum, and outer layers of the neocortex. Areas of lower than average staining intensity include: cerebellum, pons, and medulla. This regional distribution qualitatively agrees with regional CaM kinase II activity as measured by phosphorylation of exogenous Synapsin I and endogenous 50 kD and 60 kD peptides (Walaas et al., J. Neurosci., 3, 291 & 302, 1983). Light and electron microscopy show immunoreactivity within neuronal cell bodies, dendrites, dendritic spines, axons, and terminals. No immunolabeling has been detected in glial cells. Taken together, these results suggest that CaM kinase II may mediate the effects of Ca^{2+} in a number of neuronal elements and subcellular compartments. (Supported by USPHS Grants MH-17387 and NS-08440 (P.G.) and NS-15554 (P.T.K.); NSF Grant BNS-81-06259 (P.T.K.); NIMH Fellowship MH-08601 (C.C.O.); and Training Grant GM-07205 (T.L.M.).)

296.17 PURIFICATION AND CHARACTERIZATION OF BRAIN Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE I THAT PHOSPHORYLATES SYNAPSIN I. A. C. Nairn* and P. Greengard (SPON: S. I. Walaas). Dept. Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510.

Synapsin I, a synaptic vesicle-associated neuronal protein, can be phosphorylated by two distinct Ca^{2+}/calmodulin-dependent protein kinases (Huttner and Greengard, PNAS, 76, 5402, 1979; Kennedy and Greengard, PNAS, 78, 1293, 1981). The two kinases, designated Ca^{2+}/calmodulin kinases I and II, have both been purified to near homogeneity. Ca^{2+}/calmodulin kinase II was purified from rat brain (see Lai, McGuinness and Greengard, Soc. Neurosci. Abst., 1983); Ca^{2+}/calmodulin kinase I was purified, using Synapsin I as substrate, more than 450-fold from bovine brain by DEAE-cellulose and hydroxylapatite chromatography, gel filtration and Sepharose-calmodulin affinity chromatography. The molecular weight of the native enzyme was estimated to be 46,000 from gel filtration studies using Ultrogel AcA44. The purified enzyme preparation contained two major proteins, in approximately equal amounts, with molecular weights estimated to be 35,000 and 37,000, using SDS-polyacrylamide gel electrophoresis. Both proteins bound calmodulin using the ^{125}I-calmodulin overlay technique and copurified with the kinase activity through a number of chromatographic procedures. In addition, both proteins were autophosphorylated in a Ca^{2+}/calmodulin-dependent manner. The results suggest that the enzyme activity is associated with the two polypeptides of 35,000 and 37,000 daltons.

Ca^{2+}/calmodulin kinase I had a K_m for Synapsin I (Site I) of 2-3 μM, which was similar to that for cAMP-dependent protein kinase, and a K_m of approximately 30 μM for ATP. Under optimal assay conditions the purified kinase had a specific activity of 0.7 μmol/min/mg using Synapsin I as substrate. In addition to Synapsin I, Ca^{2+}/calmodulin kinase I phosphorylated smooth muscle myosin light chain. Skeletal muscle myosin light chain, glycogen synthase, tubulin and MAP-2 were not phosphorylated to any significant extent by the enzyme. (Supported by USPHS Grants MH-17387 and NS-08440.)

296.18 PURIFICATION AND CHARACTERIZATION OF BRAIN Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II THAT PHOSPHORYLATES SYNAPSIN I. Y. Lai*, T. L. McGuinness*, and P. Greengard (SPON: M. D. Browning). Dept. Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510.

Synapsin I, a synaptic vesicle-associated neuronal protein, can be phosphorylated by two distinct Ca^{2+}/calmodulin (CaM)-dependent protein kinases, CaM kinase I and CaM kinase II (Kennedy and Greengard, PNAS, 78, 1293, 1981). Both enzymes have been purified to near homogeneity (see Nairn and Greengard, Soc. Neurosci., Abst., 1983). CaM kinase II was purified from rat brain extracts by the following procedures: DEAE-cellulose chromatography, 35% ammonium sulfate precipitation, Sephacryl S400 gel filtration, and CaM-Sepharose affinity chromatography. The purified enzyme exhibited a specific activity of 4 μmol/min-mg protein when Synapsin I was used as substrate. The purified CaM kinase II had a molecular weight of 600-650,000 on gel filtration, and exhibited a major 50 kdalton (kD) peptide and a less prominent 58/61 kD peptide doublet on SDS-polyacrylamide gel electrophoresis. The 50 kD and 60 kD peptides were previously shown to be phosphorylated in a Ca^{2+}/CaM-dependent manner (Kennedy et al., J. Neurosci., 3, 818, 1983). These phosphoproteins were shown to be associated with CaM kinase II activity by a variety of methods. The peptides and the kinase activity co-eluted during chromatography on DEAE-cellulose, hydroxylapatite, phosphocellulose, dye-ligand affinity columns, CaM-Sepharose, and also during ammonium sulfate precipitation, gel filtration, sucrose density gradient centrifugation and non-denaturing gel electrophoresis. In order to examine the CaM-binding component(s) and the active site(s) of the enzyme, ^{125}I-CaM gel overlay techniques and photoaffinity labeling with [α-^{32}P] 8-azido ATP were used. Preliminary results suggest that the 50 kD and 60 kD peptides both may bind CaM and ATP. Experiments are in progress to determine whether the 50 kD and 60 kD peptides are subunits of a single enzyme or constitute different isozymes.

The substrate specificity of the purified enzyme was examined. In addition to Synapsin I, CaM kinase II readily phosphorylated microtubule-associated protein-2 (MAP-2), smooth muscle myosin light chains, and glycogen synthase. Under the conditions used, the enzyme did not phosphorylate skeletal muscle myosin light chains, phosphorylase b, tubulin, casein, or phosvitin. The ability of CaM kinase II to phosphorylate several different substrate proteins distinguishes it from the well-characterized Ca^{2+}/CaM-dependent protein kinases, myosin light chain kinase and phosphorylase kinase. CaM kinase II may be involved in several Ca^{2+} regulated functions in neuronal tissues. (Supported by USPHS Grants MH-17387 and NS-08440 (P.G.), and Training Grants GM-07324 (Y.L.) and GM-07205 (T.L.M.).)

296.19 CALCIUM/CALMODULIN-DEPENDENT PHOSPHORYLATION IN SYNAPTIC JUNCTIONS: P.T. Kelly, T.L. McGuinness* and P. Greengard. Div. of Biology, Kansas State Univ., Manhattan, KS 66506, and Dept. of Pharm., Yale Univ. Sch. Med., New Haven, CT 06510.

Synaptic junctions(SJs) from rodent forebrain prepared by the INT/Triton method were examined for Ca++/CAM-stimulated protein kinase activity and compared to synaptic plasma membrane(SPM) and postsynaptic density (PSD) fractions. The kinase(s) in synaptic fractions were examined for their ability to phosphorylate endogenous proteins or exogenous Synapsin I, in the presence or absence of calcium (Ca++) plus calmodulin (CAM). When assayed for endogenous protein phosphorylation, SJs contained approximately 25-fold greater amounts of Ca++/CAM-dependent kinase activity than did SPMs, and 5-fold more than did PSDs. When kinase activities were measured by Synapsin I phosphorylation, SJs contained 5-fold more activity than did SPMs, and 10-fold more than did PSDs. In agreement with Grab et al.(JCB, Vol. 89, 1981), the phosphorylation of SJ proteins of 60K and 50-52K(major PSD protein) was greatly stimulated by Ca++/CAM; stimulated levels of phosphorylation of these proteins were 23- and 17-fold greater than basal levels, respectively. Seven additional proteins whose phosphorylation was stimulated 6-15 fold by Ca++/CAM were identified in SJs. These proteins include Synapsin Ia and Ib, and proteins of 240K, 200K, 170K, 140K and 54K molecular weights. The 54K protein appears to be a highly phosphorylated form of the major PSD protein and the 170K protein corresponds to the Con A binding glycoprotein GP-I(Kelly and Montgomery, Brain Res., Vol. 233, 1982).

Ca++-dependent phosphorylation in SJ fractions was strictly dependent on exogenous calmodulin, even though SJs contained substantial amounts of endogenous calmodulin (15 ug calmodulin/mg SJ protein). Exogenous calmodulin, after being functionally incorporated into SJs, was rapidly removed by sequential washings. This observation suggests that the SJ-associated CAM involved in regulating Ca++-dependent protein phosphorylation may be in dynamic equilibrium with the cytoplasm.

Putative ATP binding proteins in SJs were identified using the photoaffinity analog (32P-alpha)8-azido-ATP. In SJs, four proteins were photoaffinity-labeled in a Ca++/CAM-dependent manner; two were major staining bands(60K and 52K-major PSD protein) and two were minor components(240K and 170K).

These findings indicate that a brain Ca++/CAM-dependent kinase(s) and substrate proteins are concentrated at synaptic junctions and that CAM-dependent protein phosphorylation may play an important role in synaptic functions. (Supported by NIH Grant NS-15554 and NSF grant BNS-06259 (P.T.K.); USPHS Grants MH-17387 and NS-08440 (P.G.); and Training Grant GM-07205 (T.L.M.).

296.20 HYPOTHESIS: A 47kD PHOSPHOPROTEIN (F1) SERVES AS MOLECULAR TRIGGER FOR SYNAPTIC PLASTICITY, R.F. Akers‡, S.T. Cain, G. Gonzalez-Mariscal‡, D.M. Lovinger‡, R.B. Nelson‡, and A. Routtenberg (SPON: J.P. Rosenfeld). Cresap Neuroscience Laboratory, Northwestern Univ., Evanston, Ill. 60201

The phosphoprotein F1 (MW=47kD) is altered following enhancement of synaptic reactivity in hippocampus. There is a positive correlation of F1 phosphorylation state *in vitro* that predicts the change in synaptic enhancement (Routtenberg *et al.*, Fed. Proc., 1983). These results may relate to the observed increases in F1 phosphorylation state following environmental stimulation (Routtenberg and Benson, Behav. Neur. Biol., 1980), or decreases in F1 following early neonatal handling (Cain and Routtenberg, Br. Res., 1983).

Peptides, steroids, and growth factors influence protein F1 phosphorylation. ACTH inhibits F1 phosphorylation *in vitro* (see also Zwiers *et al.*, Neurochem. Res., 1976). Moreover, we have shown that a link exists between band F1 and the opiate system: band F1 phosphorylation is correlated with opiate receptor gradients in monkey cortex (Nelson *et al.*, this volume). In the hypothalamus, progesterone-treated ovariectomized female rats exhibit increased levels of F1 phosphorylation. Finally, insulin, postulated to be synthesized in the hippocampal formation (Rosenzwieg *et al.*, PNAS, 1980), increases band F1 phosphorylation in that structure.

Since band F1 is Ca(2+)-sensitive, it is likely to be a major substrate for either the Ca(2+)-phospholipid dependent protein kinase C, or a Ca(2+)-calmodulin dependent protein kinase. Experiments are presently directed at establishing the identity of F1 kinase.

Regulation of a Ca(2+)-sensitive kinase by peptides, steroids, and growth factors would, in turn, determine the phosphorylation state of protein F1. We propose that the phosphorylation state of protein F1 thus represents a molecular switch which determines synaptic communication efficiency. Plasticity of such communication is thus under the facilitatory or inhibitory control of a diverse set of transmitter-like agents which converge on a specific kinase, with protein F1 as substrate. Supported by MH25281 to A.R.

296.21 IS THE MAJOR CALMODULIN STIMULATED PHOSPHOPROTEIN OF POST-SYNAPTIC DENSITIES A MAJOR PHOSPHOPROTEIN IN P2 MEMBRANES? John A.P. Rostas, Vicki Brent* and Peter R. Dunkley*. Neuroscience Group, Faculty of Medicine,University of Newcastle, N.S.W. 2308, Australia.

When P2 membranes are incubated with ^{32}P-ATP the phosphorylation of a number of proteins is markedly stimulated by the addition of calcium and calmodulin. The most heavily labelled protein band in P2 membranes has the same apparent molecular weight on SDS polyacrylamide gels as the major calmodulin stimulated phosphoprotein of post-synaptic densities (PSDs) which was identified as the major PSD protein (mPSDp) by Grab et al, (J. Cell Biol. 89: 440, 1981). We set out to identify this phosphoprotein in P2 membranes. The phosphoprotein is largely insoluble in Triton X-100 and deoxycholate and rendered more insoluble by pre-incubation of the membranes with p-iodonitrotetrazolium violet (INT). INT-treated P2 membranes from adult rat cortex were labelled in the presence and absence of calcium/calmodulin and synaptic plasma membrane (SPM), synaptic junction (SJ) and PSD fractions were prepared from them. The specific labelling of the calmodulin stimulated phosphoprotein was highest in SPM, SJ and PSD fractions and lowest in the mitochondrial and myelin fractions. This distribution is consistent with its localisation in PSDs. Experiments using two dimensional electrophoresis and peptide mapping after partial proteolysis are underway to determine whether the calmodulin stimulated phosphoprotein bands in the P2 membranes and the PSD consist of a single protein and whether these proteins are identical. When P2 membranes from different brain regions were labelled under identical conditions the relative amount of radioactivity incorporated into this phosphoprotein was proportional to the amount of mPSDp that is contained in SJs or PSDs prepared from the same region: incorporation in adult rat cortex was three times that in adult cerebellum and twice that in cortex from 15 day old rats. If the phosphoprotein in P2 membranes can be shown to be a single species identical to mPSDp, phosphorylation of crude membrane fractions may provide a way of measuring the amount of mPSDp in small discrete brain regions.

297.1 EMG PATTERNS IN ANTAGONIST MUSCLES ARE COUPLED TO RESPONSE DYNAMICS DURING ISOMETRIC FORCE ADJUSTMENTS IN HUMANS. J. Gordon* and C. Ghez. Dept. of Movement Sciences, Teachers College and Center for Neurobiology and Behavior, Columbia Univ. Coll. P&S, New York, NY 10032.

We have studied the activity of agonist and antagonist muscles (Bi and Tri) during isometric force adjustments at the elbow to probe the relationships between EMG patterning and force dynamics in the absence of changes in muscle length. Subjects were trained to produce accurate force pulses of different amplitudes using a visual display. They were instructed to keep force rise time as short as possible and to allow a passive return to baseline. The responses were ballistic in that the peaks of the derivatives of the trajectory were predictive of peak force ($r>.9$). Time to peak force was constant. Alternating EMG bursts in agonist and antagonist were found with a fixed temporal relationship to the dynamic features of the force trajectory. In the agonist, an initial burst (Agl) terminates at about peak dF/dt. Following a silent period one or more late bursts in the agonist occur during the falling phase of the force. With initial tonic agonist activity, an early pause precedes Agl and late agonist bursts increase in size and number. In the antagonist, there is coactivation (AntCo) during Agl, but at peak dF/dt a large reciprocal burst (AntR) occurs, lasting throughout the falling phase of dF/dt. Agl and AntR are thus closely associated with the positive and negative peaks of d^2F/dt^2. Agl (integrated EMG) varies proportionally with peak force while AntR is only weakly related.

To determine the dependence of EMG configuration on rate of rise of force, subjects were trained to produce smooth ballistic pulses to the same peak force but with a range of rise times (80-400ms). For long rise times (>200ms), agonist and coactive antagonist activity last until peak force. For intermediate rise times (120-200ms), agonist activity becomes clustered into a distinct burst ending at peak dF/dt, followed by a silent period. Only for the fastest rise times (<120ms) does AntR appear, along with late agonist bursts. AntR (integrated EMG) is then linearly related to peak dF/dt, while Agl is independent of it. Agl duration varies directly with the rise time of force. These results suggest that the temporal pattern of EMG bursts in antagonist pairs determines the stereotyped dynamics of the force trajectories of fast pulses. AntR controls and terminates the rising phase of the force trajectory rather than simply returning force to baseline. It may counteract low pass properties of muscles and non-linear summation associated with high frequency activation of motor units, thus contributing to linear scaling of peak forces in rapid pulses. Supported by the Dystonia Medical Research Foundation and NS 19205.

297.2 CHOICE REACTION TIME CONDITIONS ALTER RESPONSE DYNAMICS IN AN ISOMETRIC TRACKING TASK IN HUMANS. W. Hening, D. Vicario, and C. Ghez. Dept. of Neurology, and Ctr. Neurobiology & Behavior, Columbia Univ. Coll. P&S, New York, NY 10032.

Isometric force responses of the index finger were examined in a reaction time paradigm to determine the effect of amplitude uncertainty on the scaling and dynamics of force trajectories. Human subjects (n=4) performed a visual tracking task by exerting force on a strain gauge to match target shifts of varied amplitudes. Subjects were instructed to respond as rapidly and as soon as possible. Latencies, force trajectories and EMGs of agonist (Flex. Dig.) and antagonist (BIP) muscles were examined under two conditions of expectancy and for two instructed response profiles. Expectancy was varied by presenting different target amplitudes in a randomized series (choice conditions) or in blocks or constant size (simple condition). The required response profiles consisted of either force impulses (pulse responses) to the target, or force steps (step responses) matching and maintaining the target level.

Under simple reaction time conditions the force trajectory of both pulse and step responses was characterized by stereotyped dynamics consisting of tight linear relations between peak forces and the peaks of their first and second time derivatives. The time to achieve these peaks was independent of their magnitudes. These responses were linearly scaled to the size of target shifts and EMG activity in agonist and antagonist exhibited a triphasic alternation. Under choice conditions, the scaling of pulse responses showed saturation and non-linearities. Small and intermediate sized target shifts gave rise to hypermetric responses while large ones gave rise to hypometric responses. These responses had similar dynamics and latencies to those obtained under simple conditions. In contrast, step responses showed alterations in their dynamics under choice conditions. In these cases, the derivatives were not linearly related to the peak force and accurately scaled peak forces were then achieved by increasing the time to peak force. These responses exhibited diminished antagonist bursts and agonist activity in the silent period, providing further evidence that they were altered by updating. In sessions where brief reaction time was not stressed in the instructions, the scaling and dynamics of the responses were the same under simple and choice conditions; however, response latencies were prolonged by choice. Thus, subjects could not both respond with short latencies and maintain linear scaling without foreknowledge of the amplitude of the required response.

Supported by the Dystonia Medical Research Foundation, and NS 19205.

297.3 BRAKING OF FAST AND ACCURATE ELBOW FLEXIONS OF MONKEYS IS PROGRAMMED TO MAINTAIN SYMMETRY OF ACCELERATION AND DECELERATION. D. Flament*, J. Hore and T. Vilis. Dept. Physiology, University of Western Ontario, London, Ont. Canada, N6A 5C1.

Before studying abnormal braking of movements in cerebellar dysmetria in monkeys, it was first necessary to investigate quantitatively the braking of normal movements. For this purpose 5 Cebus monkeys were trained to make fast and accurate elbow flexions of different amplitudes (range 30^o to 60^o). These movements were made with the well-known triphasic EMG pattern in biceps and triceps.

In movements of the same amplitude performed at different velocities there was a direct linear relation between peak velocity and the peak magnitude of acceleration, the peak magnitude of deceleration and the integrated phasic antagonist burst. This indicates that symmetry was maintained between the acceleration and deceleration phases for movements of different velocities. The slopes of these relations and their intercept with the peak velocity axis were a function of movement amplitude, such that for large and small movements of the same peak velocity and the same end position: i) peak acceleration was smaller for the large movements and ii) peak deceleration and phasic antagonist activity were smaller for the large movements.

These present results are similar to the results of Marsden et al. (1) on human thumb and elbow movements and of Hoffman and Strick (2) on human wrist movements. Taken together these findings indicate that braking of fast and accurate movements of different amplitudes in monkeys and humans is centrally programmed and cannot result from simple stretch reflexes. The lower level of antagonist burst activity in large movements made at the same peak velocity as small amplitude movements has been interpreted as being due to the greater viscoelastic contribution to braking at extremes of joint rotation (1). However, this cannot be the case for the present movements which were made to the same end position. Instead we suggest that it is due to an attempt by central programming to maintain symmetry between the acceleration and deceleration phases of movements. For any given peak velocity this symmetry may minimize the energy expended in making the movement (cf. Ref. 3).

1. Marsden, C.D. et al., J. Physiol. 335:1-13, 1983
2. Hoffman, D.S. and Strick, P.L., Soc. Neurosci. Abstr. 8:733, 1982
3. Nelson, W.L., Biol. Cybern. 46:135-147, 1983

Supported by Canadian MRC and NINCDS NS17426.

297.4 THE CONTROL OF DIRECTION IN RAPID AIMING MOVEMENTS. D. C. SHAPIRO and R. A. SCHMIDT*. Motor Control Lab., UCLA, Dept. of Kinesiology, Los Angeles, CA 90024.

A current view about motor programs for rapid actions holds that the temporal structure, or relative timing, is invariant across more superficial changes in movement distance and movement time. We examined an extension of this view which predicts that the relative timing would also remain invariant across changes in movement direction, with different directions being generated by alterations in the amount of muscular activity at the participating joints.

Subjects made horizontal aiming movements of 30 cm, using a pencil-like stylus held in the right hand. Movements were at chest height, and began with the stylus positioned with the right elbow flexed 90^o. Movements directly across the body (in the frontal plane), at 45^o to the frontal plane, or in the saggital plane were conducted in separate blocks. Movement time was either 150 or 200 ms in separate blocks. EMG activity was measured in six muscles that participated in this action: anterior deltoid, medial deltoid, posterior deltoid, triceps (lateral head), biceps, and pectoralis major. Electrodes were bipolar, Ag-AgCl; EMG was recorded on FM tape, and later sampled at 1 kHz for analysis by a PDP 11/23 laboratory computer.

Although 70 trials in each movement time and movement direction condition were collected, only 30-40 trials per condition met our strict movement-time criterion of ±10% of the goal movement time. The EMG records from these trials were rectified, smoothed, and averaged across trials. These averaged EMG records revealed that the temporal structure, or pattern of activity, remained remarkably invariant across changes in movement direction. Movement direction was brought about by changes in EMG amplitude primarily, with some muscles showing changes of 100% or more as the direction changed. However, a closer analysis of the EMG bursts on individual trials revealed that, while this temporal structure is invariant in some of the muscles, in other muscles the durations and/or onset times were shifted slightly (e.g., 10-20 ms) as the direction changed.

Overall, the temporal structure (i.e., relative timing) of these rapid limb movements was largely invariant across changes in movement direction. It appears that the motor system controls movement direction by a systematic alteration in the amounts of activity in the participating muscles, leaving the temporal structure of them invariant as it does.

(Supported by UCLA Academic Senate Grant and by Grant No. BNS 80-23125 from the National Science Foundation to the second author).

297.5 THE INFLUENCE OF PRACTICE ON MOVEMENT DYNAMICS AND MUSCLE ACTIVITY. W. G. Darling* and J. D. Cooke (SPON: J. Brown), Dept. of Physiology, Univ. of Western Ontario, London, Canada.

The influence of practice on performance of elbow flexion and extension movements was studied in 16 normal human subjects using a visual step tracking task. Subjects made movements of 10 or 30 degrees amplitude to targets of 2-5 degrees width. Subjects were instructed to move accurately, without terminal oscillation and to move as fast as possible.

The first few movements made were usually slower and, in some cases, less accurate than later ones. With repetition (up to 60-90 movements) velocity increased while accuracy was maintained. The acceleration phase of the movement was relatively constant in duration after the initial movements and variability measured in groups of 10 trials decreased with practice. The deceleration phase of the movement showed a greater variability in duration which was not reduced with practice.

Movements could be performed using phasic or tonic changes in muscle EMG activity and many subjects could use both strategies to perform the movement successfully. The overall amplitude of the initial agonist and antagonist EMG bursts increased with practice as velocity increased. With practice the increased antagonist muscle activity also began earlier in relation to movement onset. In the case of flexion movements this was observed for all 3 heads of the triceps muscle.

These results suggest that the acceleration phase of the movement is programmed with practice in such a way as to minimize variations in its duration. In contrast the deceleration is more variable and, in general, appears less controlled than the acceleration phase. The EMG analysis indicates that in order to increase movement velocity while maintaining accuracy subjects increase the movement related activity of both the agonist and antagonist muscles, thus providing greater accelerative and decelerative torques.

(Supported by the Medical Research Council of Canada (MT-6699)).

297.6 EFFECTS OF ELBOW POSITION ON THE INITIAL AGONIST BURST. S. H. Brown, J. D. Cooke and K. Workman*. Dept. of Physiology, Univ. of Western Ontario, London, Canada.

It has recently been shown in this laboratory that the initial agonist burst associated with movement initiation is composed of two functionally independent components. Only the first of these components is present in small amplitude movements. Experiments were performed to determine the effect of changes in the initial length of the agonist muscle on the magnitude and duration of the initial agonist burst during small amplitude movements.

Subjects performed 10 degree step-tracking flexion movements about the right elbow. Seven different initial elbow (start) positions were examined, ranging from 65 to 115 degrees. Subjects were asked to move promptly but accurately and were allowed several minutes to practice the task before each trial began. Surface EMG activity was recorded from the biceps and the lateral head of triceps.

In most cases, little change in magnitude occurred until the initial elbow position exceeded 90 degrees at which point agonist burst magnitude increased progressively with more extended start positions. The most extended position (115 degrees) was associated with no change or a slight decrease in agonist burst magnitude. In this position there was a period of antagonist inhibition at the time of the initial agonist burst. For all positions, peak velocity and movement time were constant as was the duration of the agonist burst. In contrast to the observed dependence of agonist burst magnitude on start position, one subject showed little change in agonist activity over the entire range of start positions examined. In this case, however, antagonist inhibition was consistently observed in movements with start positions ranging from 90 to 115 degrees.

These results suggest, that for single joint movements, the magnitude of the agonist burst is dependent upon initial muscle length. In addition to changes in the phasic EMG output, modulation of tonic EMG drive may also be utilized to assist in movement initiation,

(Supported by the Medical Research Council of Canada (MT-6699)).

297.7 EMG ACTIVITY AND ARM MOVEMENT TRAJECTORIES. D. Cooke and Susan H. Brown. Dept. of Physiology, Univ. of Western Ontario, London, Ontario, Canada.

Limb movement is, in general, produced by patterned phasic drive to muscle (fast movements) or by tonic changes in muscle drive (slow movements). In addition different static limb positions (equilibrium positions) are associated with different balances or ratios of drive to opposing muscles. Experiments were performed to study 1) the effect of different patterns of EMG activity on movement trajectory and 2) the changes in ratios of EMG activities of opposing muscles during movement.

All experiments were performed on normal human subjects performing a step-tracking task using flexion-extension movements about the right elbow. Subjects moved a horizontal manipulandum pivoted at one end. Forces could be applied to the manipulandum with a torque motor. Angular position and velocity of the manipulandum (and thus of the forearm) were recorded. Surface EMG activity was recorded from the biceps and the lateral head of the triceps muscles.

When instructed to relax, subjects' arms assumed a resting or equilibrium position of approximately 80 deg elbow joint angle. Static limb positions extended relative to this position were maintained by an increased ratio of triceps to biceps EMG and positions relatively flexed by a decreased ratio. Flexion movements towards equilibrium could be made with step-like decreases in triceps activity. These movements were slow and showed terminal oscillation around the equilibrium point. Movements with phasic agonist (biceps) drive started along the same position-velocity trajectory as the slower ones but reached higher velocities. Peak velocity but not the initial trajectory was set by 1) the magnitude and the number of components of the initial agonist burst and 2) the degree of concurrent decrease in EMG activity in the antagonist. The triceps to biceps EMG ratio showed regular modulation during movement, the depth of the modulation varying with movement speed.

This data is interpreted in terms of time-dependent changes in the effective equilibrium position of the limb during movement.

(Supported by the Medical Research Council of Canada).

297.8 FACTORS INFLUENCING PERCEPTION OF VELOCITY IN RAPID VOLUNTARY MOVEMENTS. T. E. Milner*. (SPON: K. G. Pearson). Dept. of Physiology, University of Alberta, Edmonton, Canada.

The peak angular velocity (PAV) of rapid voluntary movements depends on the neural input to muscles rotating a joint, the joint angle from which movement is initiated and the forces opposing motion. The extent to which neural input was adjusted to regulate velocity when changes occurred in initial angle, movement amplitude and loading of the joint was investigated. Rapid flexion movements of the distal thumb joint were made through an angle of approximately 0.55 rad to a mechanical stop in control trials. Subjects were instructed to achieve a prescribed PAV (range: 8-20 rad/s) with knowledge of results. In experimental trials subjects were to match the PAV of control trials but were not given knowledge of results.

When the initial joint angle, movement amplitude and loading did not change between control and experimental trials, average velocity trajectories and rectified, filtered EMG profiles were very similar. The movement was initiated by a burst of EMG activity in the agonist muscle (flexor pollicis longus) while the antagonist (extensor pollicis longus) was generally silent throughout the movement.

When viscous loading was changed on experimental trials with respect to control trials, subjects varied considerably in their ability to compensate. Generally, there was a tendency to undershoot the prescribed PAV when the load was increased, but both overshooting and undershooting occurred when the load was reduced.

When subjects were instructed to initiate flexion from joint angles of greater extension or flexion in experimental than control trials, they consistently overshot or undershot the prescribed PAV respectively. This occurred even when movement amplitude was kept constant. However, when initial joint angle remained constant, but movement amplitude increased from control to experimental trials, subjects succeeded in matching control PAV.

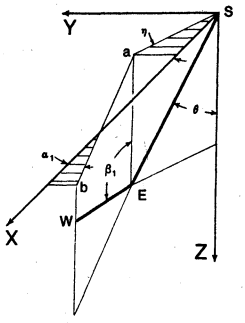
In another series of experiments subjects were instructed to begin with the thumb against the stop, to extend, then to return to the stop in one rapid motion. Linear correlations were obtained between PAV of extension or flexion and movement amplitude when subjects were instructed to move either through a prescribed amplitude or to achieve a prescribed PAV of flexion. Subjects could dramatically alter these relations when both movement amplitude and PAV were prescribed, but found the task considerably more difficult.

These results suggest that PAV is not perceived independently of initial joint angle, movement amplitude or loading.

297.9 PSYCHOPHYSICAL IDENTIFICATION OF COORDINATE REPRESENTATION OF HUMAN ARM. J. F. Soechting and B. Ross* Lab. Neurophysiology, Univ. Minnesota Medical School, Minneapolis, MN 55455.

To a first approximation, the human arm may be viewed as having four degrees of freedom: three at the shoulder and one at the elbow. There is no unique description of the angular coordinates of the arm. A number of coordinate systems may be chosen to describe arm position and in general, joint angles in each of these descriptions will not be simply related. Furthermore, there is no reason to prefer one description over another on anatomical grounds. The identification of a preferred coordinate description appears necessary, however, if one is to understand the processes by which the location of an object in extrapersonal space is mapped into joint coordinates, joint coordinates are mapped into joint torques and the processes according to which arm movements in three-dimensional space are organized and controlled.

A psychophysical approach was used to identify a possible preferred coordinate representation of the arm. Subjects were asked to match joint angles of one of the four degrees of freedom in a particular coordinate representation with their right and left arms. The right arm involved displacement in all four degrees of freedom (as in the figure below); motion of the left arm was restricted to the degree of freedom investigated in that particular experiment. Visualization was not permitted. The errors in matching joint angles in a number of different possible coordinate representations were compared, the criterion for a preferred coordinate representation being the one which led to the smallest error. Based on this criterion, we suggest the coordinate representation shown below.



S, E and W represent shoulder, elbow and wrist position, Y is lateral and Z vertical. The angles η and α are measured in the horizontal plane, θ and β in the vertical plane. Performance in matching forearm orientation β was better than that in matching the anatomical joint angle at the elbow.

Supported by USPHS NS-15018 and NSF BNS-8117625

297.10 HUMANS LACK A SENSE OF STATIC-POSITION OF THE FINGERS. F.J. Clark, R.C. Burgess* and J.W. Chapin*. Dept. of Physiol/Biophys., University of Nebraska Medical Center, Omaha, NE 68105.

Much of our information about proprioception comes from experiments with the fingers, but the sensory mechanisms for signaling position and movement of the fingers appears different from most other joints. To illustrate these differences, we examined position sense and movement sense in the proximal interphalangeal joint of the index finger and in the ankle joint. We distinguished the sense of position from that of movement by measuring how subjects' ability to detect a fixed displacement varied with the rate of joint rotation. A static-position sense should not depend on the rate of joint displacement. Therefore, if subjects possess an awareness of the static-position of a joint, slow rates of rotation should not seriously degrade their ability to sense displacements (see: Horch, K.W., Clark, F.J. & Burgess, P.R., J. Neurophysiol. 38:1436, 1975). In the absence of a static-position sense, subjects would rely on movement signals that do depend on rate of joint rotation and their ability to detect displacements should decrement with decreasing rate.

We used excursions of 3.5° for the ankle and 5° for the finger, with equal numbers of flexions, extensions and no-displacement controls (the joint oscillated a fraction of a degree returning to the start position). To count as a detection, subjects had to correctly identify the direction of a displacement. False detections remained below 5%. Our subjects could detect 80% of the slow ankle displacements with no decrement for rates as slow as 0.5°/min (the slowest tested so far). A subject's ability to sense the finger displacements began to fall at a rotation rate of about 120°/min and approached zero around 1.5°/min.

Anesthetizing the finger tip (but not skin around the rotating joint) substantially diminished a subject's ability to sense the slower rates by an amount that appeared proportional to the area of skin anesthetized. Local anesthetic injected into the joint space had no effect on the ability to detect displacements.

Blocking the common peroneal nerve at the knee to paralyze the ankle dorsiflexor muscles had little effect on a subject's ability to sense slow displacements made from a dorsiflexed position. However, with the foot set in a plantar-flexed position (to slacken the Achilles tendon) proprioception for the ankle resembled that of the finger.

We conclude that muscle receptors provide our awareness of the static-positions of the limbs, but that we lack a static-position sense for the fingers. Cutaneous receptors provide movement signals, and additionally in the fingers, cutaneous receptors even in regions of skin away from the moving joint serve an important supportive or facilitatory role in the detection of joint movement.

297.11 EVIDENCE FOR CENTRAL PROGRAMMING OF THE ANTAGONIST EMG BURST DURING RAPID VOLUNTARY MOVEMENTS. B.E. Mustard* and R.G. Lee. Dept. of Clinical Neurosciences, Univ. of Calgary Fac. of Med. Calgary, Alberta, Canada. T2N 1N4

To determine the relative contribution of central programming and segmental reflex mechanisms to the antagonist EMG burst during rapid voluntary movements, we examined EMG activity from the wrist extensors of normal human subjects in three different situations. 1) Subjects were required to perform rapid (500-600°/sec.) voluntary flexion movements of the wrist over an arc of 40° to a specified target zone. 2) Subjects were instructed to produce wrist flexions at the same velocity but with no intent to stop. These movements were terminated when the handle of the manipulandum reached a mechanical stop after about 70° flexion. 3) Passive flexion of the wrist over the 40° arc at the same velocity was accomplished by having the investigator move the handle and the subject's hand manually. For each condition, EMG activity from wrist flexors and extensors and wrist angle were averaged for two runs of 10 trials each.

Movements of the first type were accompanied by the characteristic triphasic EMG pattern with a well developed burst of activity from the antagonist (extensor) muscles occurring soon after the initial agonist burst. In the second and third conditions, the antagonist burst was either very small or completely absent. Since the extent and velocity of stretch of the extensor muscles were comparable in the three situations, we concluded that stretch reflexes account for only a small portion of the antagonist burst in this type of movement. Most of the antagonist activity is centrally programmed and appears to provide a braking mechanism when a rapid movement has to be terminated at a specified point.

To further investigate this braking function, we examined EMG patterns during rapid movements over four different distances ranging from 15° to 55°. As the amplitude of movement increased, the antagonist burst occurred later and its amplitude increased but its duration remained relatively unchanged. The effect of movement velocity was also studied by having subjects perform movements of constant amplitude (40°) at four different velocities ranging from 150°/sec. to 500°/sec. A well defined antagonist burst did not appear until the velocity exceeded approximately 250°/sec. With further increases in velocity, the antagonist burst increased in size and occurred earlier during the course of the movement. These results suggest that the timing and magnitude of the antagonist burst is related to the anticipated momentum of the moving hand as it approaches the target zone.

Supported by The Medical Research Council of Canada and The Alberta Heritage Foundation for Medical Research

297.12 BREAKDOWN IN RAPID BIMANUAL FINGER TAPPING AS A FUNCTION OF ORIENTATION AND PHASING. C.L. MacKenzie & A.E. Patla*. Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada.

This study is part of a larger series designed to investigate bimanual interaction in co-ordinated motor activity. Past research has demonstrated interaction between right and left upper limbs (Peterson, 1965; Cohen, 1970; Kelso, et al., 1979; Marteniuk & MacKenzie, 1980). This experiment was designed to investigate whether there would be alterations in the kinematics of repetitive movements of the index fingers when subjects were required to increase the frequency of these movements by keeping in time with binaurally presented clicks. Since we were interested in whether alterations in kinematics depended on spatial orientation of the hands and/or homologous muscle activation in left and right hands, we manipulated relative starting phase and the orientation of right and left hands. The subjects made simultaneous movements of the fingers either in phase (R-flex, L-flex; R-extend, L-extend) or out of phase (R-flex, L-extend; R-extend, L-flex). There were three orientations: horizontal (the fingers moved towards or away from the body midline in the transverse plane); vertical 1 (both palms were pronated and fingers moved in the sagittal plane); and vertical 2 (the left hand was supinated and right pronated and the fingers moved in the sagittal plane).

Six subjects performed three trials in the six experimental conditions. For each 32 second trial, the clicks commenced at 1 Hz and increased at .2 Hz intervals, with 8 clicks at each frequency. Subjects were instructed to move their index fingers in time with the clicks. Movements about the metacarpophalangeal joint, constrained to 20° by stops, were monitored by potentiometers. The right and left displacement records were normalized and analyzed on the HP9845 computer for deviations from the task requirements. Called "breakdowns", these deviations were defined as loss of initial phasic relationship between the two fingers. Results indicated that a "breakdown" in repetitive movements varied systematically with orientation and starting position. Specifically, "breakdowns" a) occurred earlier in out-of-phase conditions (2.9 Hz) than in-phase conditions (3.6 Hz); b) occurred later in vertical 1 conditions (3.6 Hz) than vertical 2 (3.1 Hz) and horizontal conditions (3.1 Hz); and c) occurred significantly earlier for out-of-phase starts in the horizontal orientation (2.4 Hz) than any other experimental conditions. In all the experimental conditions, the kinematics of the finger of the non-dominant hand were disrupted at "breakdown". Qualitative differences in the pattern of disruption will be further elaborated.

The inability of subjects to maintain the out-of-phase relationship between two limbs as frequency of movements increases seems analogous to the observed behaviour of animal locomotion with speed change. Supported by NSERC Grants #A8303 (CM) & #A0070 (AP).

297.13 LIMB MOVEMENT ACCURACY AND THE TRIPHASIC EMG PATTERN. P. Lagassé* and B. Keller* (Spon: M. Boulay). Physical Activity Sci. Lab., Laval Univ., Québec City, Canada G1K 7P4 and Dept. of Exercise Sci., Univ. of Massachusetts, Amherst, MA 01003.

Whereas it is relatively well accepted that movements of equal amplitude but of different difficulty levels are performed at different speeds (Fitts, 1954), the effect of target size on the triphasic electromyographic (EMG) pattern associated with rapid forearm flexion remains scarce. The present study was designed to investigate the effects of movement accuracy on the triphasic EMG pattern. During experiments, the four female and four male subjects were seated with forearm positioned horizontally and supported at the elbow while they performed maximal speed forearm flexion movements. For all experimental conditions, subjects flexed the forearm and stopped limb movement either 40 (TS40), 20 (TS20) or 10 (TS10) degrees after it reached a visual marker located 65 degrees from onset of movement. Subjects were given 200 unrecorded practice trials and 10 recorded trials for each target size. Surface electrodes placed over the biceps and triceps muscles were used to monitor EMG activity during forearm flexion. Criterion measures included start, duration and amplitude of the agonist bursts (Ag_1, Ag_2) and of the antagonist (Ant), as well as movement time (MT) for the first 65 degrees of flexion. MT was found to increase as target size decreased; it was 76, 98 and 115 msec for TS40, TS20 and TS10 respectively, which corroborate Fitts' results (1954). Ag_1 was found to be shorter during TS20 and TS10 when compared to TS40, suggesting that a longer Ag_1 is associated with a shorter MT. Since it has been established that Ag_1 is responsible for limb acceleration, this burst demonstrates longer activity time during a movement requiring less accuracy. Ag_1 EMG amplitude was greater for TS40 when compared to TS20, which demonstrates that the fastest movement is the product of the longest duration and largest amplitude, as evidenced in TS40. A larger EMG amplitude could be associated with increased recruitment of fast twitch motor units, producing faster limb movement. The onset of Ag_2 occurred earlier for TS20 and TS10 when compared to TS40 which shows that Ag_2 is related to movement accuracy as evidenced by modification of this criterion measure in the two movements requiring greater accuracy. The start of Ant was found to occur much later for TS40 than for TS20 and TS10. Since Ant is thought to provide a braking force, it must become active earlier for accuracy movements, whereas it remains silent for a longer time period during less accurate movements. The amplitude of Ant was larger during TS20 than TS40. There was no difference for this criterion measure between TS40 and TS10. It is possible that the velocity during TS10 was not great enough to require additional force from Ant to stop the movement. However, TS20 may have been fast enough to necessitate a greater braking or corrective force from the antagonist to successfully perform the movement. The results of this study support Angel's work (1980) suggesting that Ag_1 is responsible for providing the propulsive force to accelerate the limb through movement extent. Modifications in Ag_2 in movements requiring varying degrees of accuracy may indicate a relationship of this burst to the velocity of movement. Since Ant became more active as the movement necessitated greater accuracy, it can be suggested that this burst is involved in both correcting and braking the movement as previously implied.

297.14 POINTING ACCURACY IN RAPID MOVEMENTS UNDER DIFFERENTIAL VISUAL FEEDBACK CONDITIONS. D. Beaubaton and L. Hay* (SPON: J. Paillard) CNRS, INP 4, B.P. 71, 13277 Marseille Cedex 9, France.

Movements directed toward visual goals are generally subjected to a double control based on motor programming and feedback control. The respective involvement of these two processes can be greatly modified by introducing speed constraints. It seems however that some integration of visual feedback (Fb.) information is possible even during very rapid movements. This raises a question concerning the different possible types of visual control, according to velocity, with regard to the various phases of the trajectory. Pointing errors were measured for movements with an amplitude of 40 cm and different durations (from 120 ms to 270 ms), under various visual Fb. conditions : (1) Complete Fb., (2) No Fb. at all, (3) Central Fb. from the approach phase and the impact on the panel, (4) Peripheral Fb. from the first phase of movement, (5) No movement Fb., but only from the final impact on the panel. The subjects had to point a finger at 4 luminous targets randomly lit on a vertical panel including a grid-patterned printed circuit which supplied the rectangular coordinates of the pointing and the movement times. The complete, partial or absent Fb. conditions were achieved with a device in which vision of the real position of the targets and the hand (through a glass), or the virtual position of the targets (through a mirror) could be alternated or combined over the hand trajectory. The main results show : 1) a significant effect of speed and Fb. factors, 2) three levels of pointing accuracy according to Fb. conditions : the lowest under conditions involving no Fb. and with peripheral initial feedback, the highest under conditions involving complete and central Fb., and a middle level under the condition involving only Fb. of the terminal impact, 3) Under conditions involving complete, central and terminal Fb., accuracy increased with movement time, while it was not significantly influenced by movement time under conditions with only peripheral or no Fb. According to these data, 1) the role of feedforward processes is shown by the lowest level under both conditions without vision of terminal error (2 and 4) allowing an improvement of performance over the series of trials, 2) the effect of movement time on accuracy under condition 5 (terminal Fb.) suggests either a possible role of proprioceptive Fb. when movement time allows it to be integrated, or simply different levels in the quality of program execution according to time requirements, 3) there is a possibility of visual Fb. processing even during very rapid movements, as shown by the high accuracy under conditions involving complete or central Fb.. The role of visual Fb. in very rapid movements possibly consists of triggering a braking signal at the appropriate moment during ongoing movement, rather than continuously guiding it as in tracking tasks.

297.15 EFFECTS OF AGE ON PATTERNS OF MUSCLE ACTIVITY DURING AN AIMING TASK. J. Bérubé*, P. Lagassé*, C. Bard and M. Fleury. Physical Activity Sciences Laboratory, Laval University, Québec, G1K 7P4, Canada.

Whereas it is well established that important modifications occur in electromyographic (EMG) activity during the acquisition of a novel motor skill (Vorro and Hobart, 1981), the nature and extent of similar changes as a function of age still remain obscure. The purpose of this study was to investigate temporal and spatial changes in myoelectric parameters in boys of three different age groups executing an aiming task. Fifteen subjects, aged 6, 9 and 11 years, participated in this study. Each child, grasping a joy stick, performed a maximal speed horizontal arm extension in a sagittal plane in response to a visual stimulus located at eye level, 40 cm in front of him.

Surface electrodes placed on biceps, triceps, anterior and posterior deltoid were used to monitor EMG activity. For each muscle the following parameters were assessed: total reaction time (TRT), premotor (PMT) and motor (MT) times, time to peak EMG, duration of EMG activity and maximal EMG amplitude. In addition, latencies between the four muscles were calculated, as well as arm extension movement time (MVT).

The results showed that the younger subjects had the longer TRTs and MVTs. Inspection of the central and peripheral components of TRT demonstrated that longer TRTs can be solely attributed to longer PMTs. In the six year old subjects, movement was initiated by contraction of the biceps muscle whereas older subjects utilized their anterior deltoid to begin the movement. This implies that the motor program required to execute the movement has the capacity to shift from a less to a more efficient muscle during the maturity process. Duration of EMG activity in the agonist muscle was longer for the younger boys. This implies that when temporal arrangements are not optimum, the duration of electrical activity is longer. No changes were observed between groups for all parameters concerned with the role of antagonist muscles. This corroborates the generally accepted hypothesis that the activity of the antagonist muscle is the first to be regulated in the development process of the child (Gatev, 1972).

297.16 KINEMATIC FEATURES OF UNRESTRAINTED VERTICAL ARM MOVEMENTS. J. Hollerbach and C. Atkeson. Department of Psychology, Mass. Institute of Technology, Cambridge, MA. 02139.

We have investigated unrestrained human arm trajectories between point targets using a three-dimensional tracking apparatus, the Selspot System. Our studies indicate the importance of examining more natural, unrestricted movements, as our results only partly agree with previous studies of arm movement. Past observations on multi-joint human arm trajectories obtained from restricted horizontal planar movements measured with a gripped pantograph have shown in both humans and monkeys that point-to-point trajectories are essentially straight with bell-shaped velocity profiles; moreover, they satisfy a time-scaling property which may be related to the underlying dynamics. We sought to corroborate these observations for more natural unrestricted arm movements and also to examine the effects of different loads and gravity on the arm trajectories.

We measured the trajectory of the arm in human subjects using the Selspot System, which permits measurement of unrestricted movement by detection of the positions of infrared LEDs strapped onto the limb. We sampled 5 LED marker positions at a sampling rate of 315 Hz. The LED markers were attached to the shoulder, each side of the elbow, the wrist, and the tip of the first finger. For each subject, we measured trajectories of movements between pairs of targets at self-paced slow, medium and fast speeds, and repeated this procedure with weights ranging between 2 and 4 pounds held in the subject's hand.

The observations of these trajectories tightly corroborate the previous horizontal results for velocity profile and time scaling, but not for path straightness. The time scaling property holds not only across speeds but also across different hand-held loads.

Paths are generally curved, and sometimes different for upward and downward movement, between the same pairs of targets. Gravity may be used by the subject to drive the downward movements, while maintaining the appropriate time history of the trajectory. Different speed conditions and different hand-held loads do not significantly change the path of the movement; most often, the path is unchanged.

This work was supported by the National Institute of Health Research Grant AM26710, awarded by the National Institute of Arthritis, Metabolism and Digestive Diseases.

297.17 SPECIFICATION OF SPATIAL DIMENSIONS IN MOVEMENT PROGRAMMING. D. Lépine * and J. Requin. Dept. exper. Psychobiol., Inst. Neurophysiol. and Psychophysiol., C.N.R.S., Marseilles, France.

Movement-precueing methods make possible the study of information processing stages which intervene in the programing of voluntary movements (Rosenbaum, D.A. in R.A. Magill (Ed.), Memory and Control of Action, Amsterdam : North-Holland Publ. Cy, 1983). Reaction times (RT) and movement times were analyzed in a visuomanual pointing task.

Subject's wrist rotating movements controlled the displacement of two pointers in front of a vertical display panel on which 8 targets were located. They were illuminated as response signals (RS) requesting movements which differed in terms of the involved hand, movement direction (extension vs flexion) and movement extent (short vs large). Three seconds before the RS, either no information or information about either 1, 2 or all movement dimension(s) was given to the subject by illuminating for a 1 sec period a set of either 8, 4, 2 or 1 LED(s) disposed next to the targets.

Data show 1) that contrary to the hypothesis of Goodman and Kelso (J. exper. Psychol.: General, 1980, 109 : 475-495), RTs observed in this high stimulus-response spatial compatibility condition do not depend upon event uncertainty only, i.e. upon the number of responses between which the subject had to choose after the RS occurred. It can be concluded, as suggested by Rosenbaum (J. exper. Psychol.: General, 1980, 109 : 444-474) and Bonnet, Requin and Stelmach (Bull. Psychon. Soc., 1982, 19 : 31-34), that response programming proceeds through distinct specification of the different movement dimensions, 2) that dimension specification is not systematically processed in a serial order. This latter would imply an additive relationship between RTs observed in some of the precueing conditions, that which was not confirmed.

Results can be interpreted within the frame of a general model based upon 1) a parameter for signal processing, 2) a parameter for response programming specification which depends upon the nature and features of the movement dimensions for which advance information is provided. The viability of some simplifying hypotheses about the relationships between specification times, i.e., on the programming process itself, is evaluated.

297.18 HISTORY OF THE EARLY MEETINGS OF THE AXONOLOGISTS. L.H. Marshall. Neuroscience History Resource Project, Brain Research Institute, Univ. of California, Los Angeles, CA 90024.

The axonologists met for the first time on March 26, 1930 at the invitation of Ralph Gerard. He stated that he invited "everyone working on nerve who was present at the meeting of the American Physiological Society that year" (1, p. 467) to join him at dinner and an evening of discussion before the annual meeting, held in Chicago. More than fifty years later, little is known about that and subsequent meetings because no records were kept and there was minimal formal organization. There is more or less agreement that 11 American axonologists partook of dinner at the University of Chicago's Quadrangle Club and proceeded to Gerard's home afterward. Yet the APS program listed about 40 papers on brain and nervous system. Did Gerard exercise a certain selectivity in issuing his invitations? Against this conclusion is the fact that there had been a heavy snowstorm, so perhaps some invitees were prevented from attending. Also, the invitation sent to Detlev Bronk, the only copy that has surfaced to date, lists the names of 15 investigators "in nerve" who received invitations. Of the American axonologists at the first meeting, all but one in subsequent years were elected to the National Academy of Sciences, and eight became heads of departments or institutes. Whether or not Gerard planned it that way, the original axonologists were an elite group.

William A.H. Rushton was one of the visitors from abroad who attended the Chicago meeting. He wrote that "This experience was a great enlargement for me" (1, p. 280). Rushton also says that Ali Monnier and Ragnar Granit were at that meeting.

Apparently that first evening the discussion was general, but by the second gathering, a luncheon in Montreal, the topic was nerve conduction. In 1932 the group at a dinner in Philadelphia had swelled to about 35 axonologists and a committee (of super-axonologists) had to be formed to continue. The question of a journal of neurophysiology was raised, but Joseph Erlanger, the patriarch of the group, was against splintering. In 1933 there were two meetings on separate days, one for business, the other for a roundtable on cerebral action potentials. Gerard was again organizer in 1934 when the axonologists met in New York and the talk was on "single-fiber axon action potentials." The axonology meetings were now so large that they became more formal and in a few years were abandoned altogether.

1. F.G. Worden, J.P. Swazey, and G. Adelman, Eds., The Neurosciences: Paths of Discovery. Cambridge, Massachusetts, MIT Press. 1975.

DISORDERS OF THE MOTOR SYSTEM: NEURAL PROSTHESES

298.1 COLLAGENASE ACTIVITY IN SKIN FIBROBLASTS AND EXPLANTS OF ALS PATIENTS Robert L. Beach, Erlinda T. Reyes, Janet Schoeling*, Heinz Popiela and Barry W. Festoff Neurobiology Research Lab V.A. Medical Center and Dept. Neurology, Univ. Kansas College of Health Sciences Kansas City, MO 64128,

In our efforts to clarify previous evidence of enhanced collagenolytic activity and connective tissue abnormalities in skin of ALS patients (Neurol. 10:717, 1960; Lancet 1:1007, 1966), we have measured the collagenolytic activity released into media by skin fibroblasts and biopsies from ALS patients and controls. Released collagenase activity is not elevated in our relatively small sample of ALS patients skin fibroblasts. In fact, there is significantly less collagenase released by fibroblasts from ALS patient biopsies, but only when values are adjusted for protein released. These values are the total (active + inactive) collagenase activity in the media since we activated the samples with p-amino-phenylmercuric acetate (APMA). We also assayed 10 fold concentrated samples of media in the absence of APMA to detect the very low levels of collagenase in media which are already present as the active enzyme. Again ALS patient's fibroblasts had lower collagenase activity than controls, but only when adjusted for protein released. The percent of collagenase enzyme which was active (ie. without APMA activation) in the media was similar for ALS patients and control fibroblasts. The protein released by ALS patient skin fibroblasts is higher than controls, but the group sizes are small, so it is premature to conclude that there is a significant alteration in release of collagenase by cultured human skin fibroblasts. We have recently analyzed the release of collagenase from explant cultures of the skin biopsies. There were no signifigant differences in the release of collagenase from ALS patients' skin explants relative to normal control explants during the first 27 hrs of incubation. We also assayed the release of collagenase from long term organ cultures of the skin biopsies and found that there was a dramatic increase in collagenase activity released after six days in organ culture. The increase in activity was from background levels of activity to very high levels. These results have been obtained on explants from ALS patients. These results are consistent with the previously reported increase in collagenolytic activity in ALS patients. The relationship between increased collagenase activity and disease progression in ALS victims is under investigation.

Supported by grants from ALSSOA, NIH (RO1 NS 17197) and the Kansas University ALS Regional Research Center.

298.2 ENVIRONMENTALLY INDUCED CIRCLING IN CATS. W. G. Christen*, G. D. Mower and F. H. Duffy. Dept. of Neurology, Children's Hospital, Boston, Mass. 02115.

Administration of dopamine agonists to rats with unilateral lesions of the nigro-striatal pathway induces circling behavior. The structures mediating this behavior are still in question. One of the problems in identifying the output stations for this behavior is that the behavior itself, as well as the destruction of potential output stations, is produced by invasive techniques. The extent and localization of lesions can be difficult to control.

In the course of our investigations on the etiology of amblyopia we have found that environmental manipulation is capable of inducing circling in cats. We have manipulated the visual environment of young kittens by rearing them under conditions of unequal alternating monocular deprivation. Three cats have been reared in the dark until 4 weeks of age at which time visual exposure sessions were initiated. The animals received 2 hrs. of monocular visual input daily (an opaque occluder was placed in the non-viewing eye) after which they were returned to the dark. Only one eye received visual exposure on any one day. One animal was reared with a ratio of 6 days of vision for the more experienced eye (MEE) for every 2 days of vision to the lesser experienced eye (LEE). The other 2 cats were reared with a ratio of 7/1.

By 6 months of age all 3 cats were rapidly spinning in tight circles (approx. 60 revolutions/minute) for most of the 2 hr. visual sessions. Two of the cats (one of each ratio) circled in a direction contralateral to the LEE, and only when this eye was open. Behavior with the MEE appeared normal. The remaining cat circled in a direction ipsilateral to the LEE regardless of which eye was open. Cats reared with less extreme (4/4, 5/3) and more extreme (100/0) ratios failed to develop this behavior.

These results indicate that the manipulation of a developing sensory system can produce a motor asymmetry that has previously been produced only by invasive techniques. The results also suggest that visual nuclei or pathways are involved in the expression of nigro-striatal motor function.

298.3 UNIFORMITY OF PARKINSONIAN TREMOR IN THE OROFACIAL AND DIGITAL MOTOR SYSTEMS. C. J. Hunker,* J. H. Abbs* (SPON: R. Goldstein). Speech Motor Control Labs., Waisman Center, University of Wisconsin, Madison, WI 53706.

Despite a large number of clinical and basic studies, the neural correlates of the various pathological tremors in Parkinson's disease remain at issue. These tremor forms have considerably lower frequencies, larger amplitudes, and different activation modes than normal physiological tremor. These features of parkinsonian tremors have been variously attributed to certain supraspinal influences, aberrant spinal networks (i.e., stretch reflex arc, recurrent inhibition-rebound), etc. However, a number of classical spinal neuromuscular circuits such as those underlying muscle spindle actions do not operate in the lip and tongue muscles and recurrent or reciprocal inhibitory networks apparently are not present in the cranial motor nerves. In this regard, the biomechanical and neurophysiological differences among the various spinal and cranial motor systems provide a useful basis on which to examine the tremorgenic theories formulated from studies which have focused exclusively on the limbs.

Six parkinsonian adult males with a prominent tremor component served as subjects. Tremor was examined in the lips, tongue, jaw, and index finger from measures of contractile force and agonistic muscle EMG during targeted low level isometric contractions. Spectral analyses revealed that the force tremor modal frequencies were remarkably consistent across the orofacial and digital motor systems. Two subject-specific tremor patterns with characteristic modal frequencies and spectral shapes emerged. One subgroup of Parkinson subjects showed large amplitude, sharply tuned tremor peaks at 4.0-5.0 Hz while a second group showed low amplitude, broadly tuned peaks at 8.0-10.0 Hz. A uniformity in tremor manifestation in the digits and orofacial structures seriously challenges hypothesized neuromuscular explanations based upon spinal-specific mechanisms and argues for a central tremor source. Seemingly, the neural pathways responsible for parkinsonian tremor must be common to both the cranial and spinal motor systems. Research supported by NIH grants NS-13274-07 and 5-P30-HD-03352-14.

298.4 CLONIC OSCILLATIONS OF THE LOWER LIMB. Paul A. Iaizzo and Robert S. Pozos (SPON: R. J. Ziegler). Dept. of Physiology, Univ. of Minnesota, Duluth, School of Medicine, Duluth, MN 55812.

The mechanism and maintenance of clonus remains controversial, hence the impetus for this study. Two different clonic oscillations were elicited in the lower limb of spinal cord patients. By analyzing and comparing these oscillations a better understanding of their mechanisms may be obtained.

A modified procedure was used to elicit ankle clonus (AC). The patients were seated with their knees flexed to form 90° angles while their feet were resting flat on the floor. Their lower legs were then passively lifted to a plantar flexed position and then allowed to passively fall to the floor; this was sufficient stimuli to initiate AC. Weights were added to each patient's limb and changes in AC were recorded. Clonic oscillations of the whole lower limb (limb clonus, LC) were recorded in one seated spinal cord patient who had a complete traumatic lesion at T 5-6. LC was initiated by the patient extending and stretching his lower back. His knee involuntarily became fully extended for 4-5 seconds during the time which the LC was recorded. All clonic oscillations were detected with an AVR-250 accelerometer taped slightly proximal to the patient's patella. Using biopotential surface electrodes, EMG's were detected from various leg extensor and flexor muscles. Acceleration and EMG signals were recorded on a FM tape recorder and later analyzed using a MINC-11 computer.

Analysis of the data show all clonic oscillations fall within the same frequency range of 5-7 Hz. The EMG activity which is highly correlated with the AC (coherence values $> .9$) is limited to the extensor muscles of the lower leg. However, EMG activity highly correlated with LC can be observed in extensor muscles throughout the leg (i.e., soleus and quadraceps). In both AC and LC, there is poorly correlated EMG activity of the flexor musculature with motion. The addition of mass altered the frequency and amplitude characteristics of the acceleration and EMG signals recorded during AC.

The clonic-like oscillations of the ankle in normal subjects have been attributed to mechanical-reflex factors (Stiles and Rietz, Am. J. Physiol., 233(1):R8-R14, 1977) and the data reported here is in support of similar factors controlling pathologic clonus. The strong correlation between the various extensor muscular activity during LC may be explained by the clonic activity in one extensor group (i.e., quadraceps) influencing the excitability of motoneurons of other leg extensors (i.e., soleus), by intersegmental connections.

298.5 RESPONSE OF TONIC MUSCLES INNERVATED BY NERVES OF TWITCH MUSCLES IN NORMAL AND DYSTROPHIC CHICKENS. A.C. Gandy* and E. Cosmos. Dept. of Neurosciences, McMaster Univ. Health Sci. Ctr.,Hamilton, Ontario L8S 3Z5.

In chickens afflicted with hereditary muscular dystrophy, only fast twitch (FT) muscles express disease phenotypes; slow tonic (ST) muscles are spared disease characteristics. Thus, although genotypically dissimilar, ST muscles of normal (N) and dystrophic (D) lineage are phenotypically similar. As a result of these observations, we questioned whether ST muscles of the two genotypes would respond similarly when coupled to a nerve from a FT muscle. Cross-reinnervation experiments were performed on newly hatched N (n=50) and D (n=50) chicks. Using the method of Zelená and Jirmanová (Exp. Neurol. 38:272,1973), the ST anterior latissimus dorsi (ALD) muscle was coupled to the predominantly 'fast' superior brachialis nerve. Parameters used to monitor the response of the ALD muscles to the foreign nerve included physiological, histoenzymic, and quantitative structural analyses performed from 2 to 32 weeks postoperatively.

Typically, unoperated ALD muscles exhibit multiple innervation and do not respond to a single indirect stimulus in vivo. Upon examination, focal endplates were consistently observed in cross-reinnervated (X-RI) muscles of both genotypes. In addition, since X-RI muscles of both genotypes twitched in response to an indirect single supramaximal stimulus, we concluded that these muscles accepted and responded to a foreign nerve.

To determine the degree of responsiveness of genotypically N vs D X-RI ALD muscles, the histochemical myosin ATPase reaction was employed. Unoperated ST ALD fibres demonstrate dual acid and alkali stable myosin ATPase activity whereas unoperated avian FT fibres exhibit alkali stability only. Thus, with this parameter, we could determine both the percentage of individual fibres which responded to the foreign nerve and their pattern of distribution within X-RI muscles. As early as 2 weeks, X-RI muscles of either genotype contained fibres which displayed alkali stable activity only. Between 2 and 32 weeks, the X-RI muscles of D birds consistently exhibited a greater response to the transposed foreign nerve than did X-RI N muscles; the percent conversion was maintained at a higher level in the X-RI muscles of D birds. Over 80% of all X-RI D muscles demonstrated a degree of conversion $>40\%$; only 37% of X-RI N muscles showed a comparable degree of conversion. Further, the distribution pattern of converted fibres was relatively consistent in all of the X-RI D muscles but demonstrated a wide variability in X-RI N muscles. Therefore, our observations indicate that ALD muscles of dystrophic and normal genotypes do not respond similarly to the experimental alteration of cross-reinnervation by a foreign nerve.
(Supported by MDAC and MDA)

298.6 ACETYLCHOLINESTERASE AND ITS MULTIPLE MOLECULAR FORMS IN EMBRYONIC MUSCLE FROM NORMAL AND MUSCULAR DYSGENESIS (mdg/mdg) MOUSE. M. Pinçon-Raymond*, L.H. Tran* and F. Rieger. Unité de Biologie et Pathologie Neuromusculaires. INSERM U 153. 17, rue du Fer-à-Moulin 75005 Paris, France.

Acetylcholinesterase (AChE) of normal mouse embryonic muscle is composed of several active molecular forms, identified after detergent and high salt extraction, by sucrose gradient sedimentation analysis. As in the rat embryo, the tailed, asymmetric 16 S form is present as soon as the first contacts between the growing axons and myotubes are observed at the electron microscope level, i.e. at embryonic day 13. In mdg/mdg muscle, the relative proportion of 16 S AChE relative to the other forms is lower than in normal muscle and remains low during the embryonic development, (Rieger, F., Powell, J., Pinçon-Raymond, M., Dev. Biol., 1983, in press). Two important ultrastructural abnormalities may be related to this deficit : the immaturity, and even the lack, of the T. tubule system and the abnormal formation of muscle basal lamina. In the diaphragm muscle, the ultrastructural analysis shows that the mdg/mdg myotube does not form mature contacts between the sarcoplasmic reticulum and T. tubules (diads, triads), and acquire later than the normal myotube a less dense lamina. The first structural abnormality is probably directly involved in the lack of muscle contractile activity in mdg/mdg muscle which in turn may decrease 16 S AChE biosynthesis and the second one suggest a possible depletion in the basal lamina structures, which preferentially retain and/or attach hydrophilic 16 S AChE. We further evaluated the pools of 16 S AChE which are associated to muscle basal lamina or to the muscle plasmic membrane itself by differential, step-by-step (high salt-detergent sequences) extraction. Most of the mdg/mdg 16 S AChE is associated to the plasmic membrane, which suggests that the ultrastructural basal lamina abnormality leads to a major cellular inability in incorporating or accumulating 16 S AChE in its normal privileged extracellular location.

298.7 Pharmacological Manipulation of the Motor Symptoms Exhibited by the Spastic Mutant Mouse. J. J. Cordon*, J. M. Weaver*, P. J. Mickevicius* and F. W. Marcoux. (SPON: C. Taylor) Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

Although the "spastic" mutant mouse was described in 1960, its pathophysiological relevance to clinical spasticity remains unclear. These mice exhibit tremor of limbs and tail, which is exacerbated by startling, and difficulty in regaining upright posture when placed on their backs. In addition, recent work with the spastic mouse has demonstrated abnormal EMG patterns when compared to unaffected litter mates.

We have attempted to characterize pharmacologically the "spastic" symptoms exhibited by the spastic mutant mouse at 16 or more weeks of age. Drug standards were tested for their effect on time to regain upright posture after the spastic mouse was placed on its back (righting time). To determine adversely effective dose levels, control, nonspastic mice were dosed and evaluated as to their ability to cling to an inverted platform screen; inability to cling to the inverted screen for 60 seconds was interpreted as a toxic effect. Drugs were administered to spastic mice in 3 dose levels (IP) and righting time was evaluated at 30 minutes, 2 hours and 4 hours later. Drugs were evaluated for toxic effects in nonspastic mice on the inverted screen at the same doses by the same schedule.

Diazepam, baclofen and dantrolene sodium were chosen as drugs commonly prescribed for spasticity in patients. Diazepam reduced righting time at 1, 3 and 10 mg/kg when evaluated at 30 minutes, 2 hours and 4 hours after treatment; failure to cling to the inverted screen (toxicity) was observed at 3 and 10 mg/kg. Baclofen reduced righting time at 10 mg/kg when evaluated at 2 hours and 4 hours after treatment; this dose was toxic at 30 minutes, but not 2 hours and 4 hours after treatment. Dantrolene sodium at 30 mg/kg reduced righting time at 2 and 4 hours and was not toxic. Pentobarbital, amphetamine and morphine were chosen as representatives of other drug classes. Pentobarbital reduced righting time at 30 mg/kg when evaluated at 30 minutes after treatment, when it was also toxic. Amphetamine tended to reduce righting time at .3, 1 and 3 mg/kg when examined 4 hours after treatment, and these doses were not toxic. Morphine reduced righting time at 3, 10 and 30 mg/kg when evaluated at 30 minutes after treatment, but not at 2 hours and 4 hours, and was not toxic.

These data suggest that a pharmacological reduction of righting time in the spastic mutant mouse is consistent with a reduction in muscle tone.

298.8 THE RELATIONSHIP BETWEEN THE DISTRIBUTION OF DAMAGED AXONS AND NEUROLOGIC DEFICIT IN A RAT MODEL OF SPINAL CORD INJURY. W. Stewart, T. Kaczmar*, W. Collins and C. C. LaMotte, Sect. of Anatomy, Neurosurgery, and Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510

Since HRP is known to be taken up by damaged axons, we have exploited this phenomena to examine the distribution of damaged axons in the contused rat spinal cord. Rats were subjected to impact injury at T6. Three doses of injury were employed using the weight drop method, 2 mg X 20 cm, 4 gm X 10 cm and 5 gm X 8 cm. Immediately following injury the dura was opened and a HRP-gel strip laid on the dorsal surface of the cord at the site of impact. After a 2 hr. survival the rat was perfused and the cord removed, embedded in paraffin and sectioned transversely. Sections were pretreated with methanol to inactivate endogenous peroxidase and then the PAP method was used with an antibody to HRP. The distribution of labelled fibers was drawn with the aid of a microscope and camera lucida.

In a parallel series of chronic experiments rats were examined neurologically following these impact doses. We have used the tilted plane method (Rivlin and Tator, Surg. Neurol. 10:39, 1978) to test the motor status of the rats.

Rats subjected to the 2 X 20 dose were transiently paraplegic. Their scores on the tilted plane test returned to control levels by two weeks after trauma. The HRP results indicated a substantial rim of white matter that had few labelled axons. By contrast the rats in the 4 X 10 and 5 X 8 groups had low motor scores at one and two weeks following trauma. The HRP results indicated damaged axons throughout most of the white matter.

We conclude that: 1) The HRP-PAP method is valuable for demonstrating axons damaged at or soon after impact trauma; 2) There is a positive correlation between the extent of damaged fibers immediately following injury and the resultant neurologic status and 3) Since all these doses would be termed 40 gm - cm by the traditional method and since they produce varying degrees of injury, the simple method of multiplying weight times height to calculate dose should be discarded.

Supported by NS10174.

298.9 BIOENGINEERING CHANGES IN SPASTIC CEREBRAL PALSY GROUPS FOLLOWING CEREBELLAR STIMULATION. R. Davis, E. Gray*, T. Ryan*. Dept. Neurosurgey, Mt.Sinai Medical Center, Miami Beach, FL. 33140; Dept. Biomed. Eng.,Univ. Miami, Coral Gabels, FL. 33100

Quantative bioengineering tests were performed on 30 spastic cerebral palsy (CP) patients who underwent chronic cerebellar stimulation (CCS) from May 1979. The twin pad electrodes were placed on the superior paravermal area, and connected to a fully implantable pulse generator (1.0-1.4 milliamp, 0.5 msec, 150 pps, 4 min. ON/OFF), the charge density is 1.1-1.8 uC/sqcm/ph. In more severely graded patients, respiratory inductive plethysmography was used to measure 9 pts. of which 8 had paroxysmal and/or ataxic breathing patterns, 5 pts. were shown to revert to normal patterns with the 3 others markedly improved, within 5 mos. of CCS. Using an oscillator foot pedal (1-10Hz) compliance testing was performed on 4 very spastic pts. who showed improvements in 9 of the 16 tests performed.

In the midly-moderately spastic CP groups, 17 patients' performance ability was evaluated with 9 comprehensive tests; following one week of CCS 52% showed performance increases greater than 10%, increasing to 62% during the first year (average 6.3 mos.). Specifically (1st week: 1st year results), complex visual motor testing (20%, N=8: 31%, N=3), static strength (32%, N=13: 73%, N=9), finger dexterity (12%, N=8: 7% decrease, N=4), and gross arm movements (8%, N=15: 27%, N=9) were performed. Tracting showed improvements in error, velocity and reaction time (1-31%, N=6: 7-24%, N=2), all 5 pts. analyzed for gait change showed increases in co-ordination index. Performance testing shows that 40% of the pts. were markedly improved in the 1st week of CCS, while 60% reached this level after 6 months. A "blind" study showed discernible changes with ON/OFF testing during the first three months, but little change at 6 months.

298.10 TOPICAL ANESTHESIA: CHANGES IN THE CONTROL OF MOVEMENTS IN SPASTIC PATIENTS, M.A. Sabbahi, S. Roy*, C.J. De Luca, and L. VanVolkinberg*, NeuroMuscular Research Lab, Dept. of Orthopaedic Surgery, Children's Hospital, Harvard Medical School, Boston, MA 02115 and Liberty Mutual Res. Ctr., Hopkinton, MA, 01748

Cutaneous receptor afferent discharges have been shown to significantly affect the modulation of the soleus motoneuron pool (MNP) (Sabbahi and De Luca, 1981, 1982). The question addressed here is whether this modulation of MNP excitability results in change of the movement behavior in patients affected with spasticity.

Patients with chronic spasticity were tested. Measurements consisted of the temporal components of the gait cycle, the walking speed, time to perform reciprocal movements at the elbow and knee joints, and the active and passive range of movements of the upper and lower limb joints. Modification in these measurements were related to the improvement of the active movement patterns recorded by videotape. Measurements were recorded before and after application of either topical anesthesia or a placebo to the skin of the affected limbs. Patients were selected randomly to receive a physical therapy program combined with either the topical anesthetic or a placebo spray for one month. In the second month of the treatment program, the spray was changed so that each patient was administered an active and a placebo spray during an equal amount of time. At the end of the two-month period, the tests were repeated and the changes were calculated.

Results show a substantial shift, towards normalcy, in the control of movements of the upper and lower limbs post-anesthesia. The degree of changes in movement post-anesthesia varies between the upper and the lower limbs and between patients according to the location and extent of the lesion. Step times were more symmetrical, support times on the affected limb were longer, double support times were shorter and walking speed was faster post-anesthesia. Time to perform fast reciprocal movements at the elbow and knee joints was shorter; passive range of the distal and passive and active range of proximal joints substantially increased post-anesthesia. No measurable changes were recorded post-placebo.

These immediate changes in movement behavior were maintained or increased after the one-month period of anesthesia, thereby, improving the functional ability of the affected limbs. No measurable changes were noticed after the one-month period of placebo.

These findings imply that reduction of cutaneous receptor afferent discharges modulate the MNP excitability resulting in a substantial increase in the control of movement which could be useful in treatment of spastic patients.

(Supported by Liberty Mutual Research Center)

298.11 EFFECTS OF DIFFERENT STIMULATION PARADIGMS ON PHYSIOLOGICAL AND HISTOCHEMICAL PROPERTIES OF STIMULATED MUSCLE. H.E. Stone*, A.S. Ferguson*, J.T. Mortimer and U. Roessmann (SPON: L.F. Dell'Osso). Applied Neural Control Lab., Dept. of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106.

Electrical stimulation is known to alter physiological and metabolic properties of mixed-fiber type muscles. Although many studies have employed a wide variety of rates and patterns of stimulation, no research compares the effects of different stimulus paradigms on contralateral muscles of the same animal. The purpose of this study is to compare physiological and histochemical changes in electrically stimulated muscle resulting from two different stimulation paradigms applied to contralateral muscles in the same animal.

Tibialis anterior muscles of cat hind limbs were stimulated supramaximally for 90 days, 24 hours/day, using 100 usec balanced biphasic current pulses. One limb was continuously stimulated at 10 Hz ; the other limb received a 30 Hz burst paradigm, consisting of a 2 second frequency ramp (5 Hz to 30 Hz), 2 seconds at 30 Hz, and an off-period of 5.7 seconds. Both paradigms were chosen to deliver the same number of pulses, (i.e. 97 pulses per 9.7 seconds or an average frequency of 10 Hz).

Histochemical results indicate both stimulation paradigms resulted in muscles with uniform fiber types; fibers stained dark for NADH (indicating high oxidative enzyme activity) and stained light for myofibrillar ATPase. There was a tendency for hypertrophy of the fibers stimulated with the 30 Hz burst paradigm.

Physiological data showed slower contraction times for both muscles, although the 30 Hz muscle was significantly faster than the 10 Hz muscle. The 30 Hz muscle also had a lower twitch force but larger tetanic tension, resulting in a smaller twitch/tetanus ratio than that found for the 10 Hz muscle.

Following one hour of stimulation at their respective paradigms, the 30 Hz muscle showed a 5-10% fall in peak force, while the 10 Hz muscle had a 30-50% fall in peak force. Integration of the isometric force over time yields a larger value for the 30 Hz muscle, indicating this paradigm is more effective in adapting muscle to applying force for longer periods of time. The 30 Hz paradigm could be useful for altering bone structure as may be required for correction of a scoliotic spine.

Supported by NIH Grant No. 5 R01 AM27878-03 and NIH Training Grant No. HL075-35.

298.12 A MODIFIED BIPOLAR CUFF ELECTRODE FOR COLLISION BLOCK OF PERIPHERAL NERVE. J.D. Sweeney* and J.T. Mortimer. Applied Neural Control Lab., Dept. of Biomedical Engineering, Case Western Reserve Univ., Cleveland, Oh. 44106.

A new type of bipolar cuff electrode has been developed to affect unidirectionally propagated action potentials (UPAP). Previous work at this laboratory has shown that antidromic UPAP can be used experimentally to block unwanted naturally or artificially occuring orthodromic neural activity. This "collision block" occurs when the antidromic UPAP collide with the orthodromic action potentials to cause mutual annihilation. A collision block of motor signals in the pudendal nerves could be used to control spasticity in the external urinary sphincter that has hindered efforts to develop a bladder evacuation neuroprosthesis for spinal cord injured subjects.

A conventional bipolar cuff electrode with the anode located distally and the cathode proximally can be used for generation of antidromic UPAP if orthodromic conduction currents are opposed by sufficient anodic current flow. At pulse widths and current amplitudes required to produce antidromic UPAP, however, current flowing external to the cuff can produce excitation at a virtual cathode distal to the anodic conduction block.

A modified bipolar electrode has been designed that can generate antidromic UPAP without virtual cathode excitation. This new electrode differs from a standard bipolar cuff electrode in that the anode is placed within an insulating sheath of larger diameter than the cuff's cathode. In all 13 acutely studied cats modified bipolar cuff electrodes with stainless steel anodes (1.6 or 3.4 mm diameter) and cathodes (1.2 mm diameter) produced positive block windows (i.e. ranges of current over which UPAP were generated) when used in an elastomer rubber cuff containing the medial gastrocnemius muscular branch of the sciatic nerve (approximately 1 mm in diameter). The cuff length used was 16 mm while cuff length asymmetry (i.e. distance from cathode to proximal end over distance from cathode to distal end) was 1.7 to 1. Stimuli were regulated-current monophasic rectangular pulses with exponential trailing phases. With pulse widths within the range of 100 to 500 microseconds exponential fall times of 100 to 500 microseconds could be chosen that minimized total charge injection for a given current amplitude. Virtual cathode excitation was always adequately suppressed. Modified bipolar cuff electrodes also produced larger block windows than monopolar stimulation with the same insulating cuff.

Supported by NSF Grant No. PFR80-17190 and NIH Training Grant No HL075-35.

298.13 SUBDERMAL IMPLANTED ELECTRODES FOR ELECTROCUTANEOUS SENSORY FEEDBACK IN UPPER EXTREMITY FES ORTHOSES. R.R. Riso, K.R. Gates*, T.R. Ignagni*, M.W. Keith and W.D. Williams*. Rehabilitation Engineering Program, Case Western Reserve Univ. Cleveland, Ohio.

Functional Electrical Stimulation (FES) techniques hold promise for providing a practical method of restoring functional grasp in persons having C5 and C6 level spinal injuries. The usefulness of the prehension provided is compromised, however, by the lack of tactile and kinesthetic sensibility, and control is mainly guided by visual feedback which can be burdensome. By applying coded electrical stimuli to a skin area where sensation is unimpaired, substitute sensory information can be provided. Applications of this schema in upper extremity rehabilitation by several investigators have centered around myoelectric prostheses, where the stimulating electrode may be incorporated into the shell of the prosthesis to make contact with the skin of the amputee's stump.

Such an arrangement would be incompatible with the design of future generation FES orthoses which will utilize implantable components to minimize external hardware and thereby maximize cosmetic acceptability. As an alternative,we have demonstrated the feasibility of eliciting electrocutaneous sensations by means of percutaneous, monopolar, fine coiled stainless steel wire electrodes, implanted using a hypodermic needle to lie just beneath the skin in the region of the upper arm. Many of these electrodes have been allowed to remain in place for months while protracted studies were performed. Experiences from 7 subjects who collectively received 16 electrodes show that single or bursts of monophasic, capacitively coupled,rectangular stimuli (10-100 usec pulse width; 2-100 Hz) consistently elicit clear distinct sensations localized to the electrode site. An evaluation and comparison of subjects' abilities to make discriminations of pulse freq. (range 2-100 Hz) using subdermal vs. surface electrodes, showed that the sensations induced by the subdermal stimulation were more comfortable and distinct, and less accommodation took place than was the case with the surface stimulation. Above 20 Hz, JNDs were smaller when the stimulation was applied subdermally. Threshold currents differed from one electrode to another (range 0.3-6.0ma; mean= 1.4) but for any given electrode remained reasonably constant. The ratio of the current level that was painful to that which was just detectable was typically within in the range of 3-5.

A tracking task designed to evaluate the fidelity of freq. modulation codes revealed that increases in freq. are more readily perceived than are decreases and that abrupt decreases produce subjective momentary suppressions of sensation. In a comparison of the use of single pulses vs. using bursts of 6 pulses it was shown that a superior code results when the # of pulses in each burst is made to vary with the burst repetition freq. presented.

(Supported by NIHR grant #G001005815).

299 **SYMPOSIUM. CLOCKS IN THE TEST TUBE: TOWARD A MECHANISTIC ANALYSIS OF CIRCADIAN OSCILLATORS.** M. Menaker, Univ. of Oregon (Chairman); G. Block, Univ. of Virginia; A. Eskin, Univ. of Houston; J. S. Takahashi, Northwestern Univ.

Several organs that show circadian properties have been isolated from both invertebrates and vertebrates. Some of these can be maintained in vitro for up to 10 cycles and the properties of the clocks that they contain can be studied in the absence of their normal complicating interactions with the rest of the organism. In this symposium we will review the progress that has been made in understanding the generation of circadian oscillations at the organ, cell and biochemical levels by the study of these in vitro systems. Further, we will attempt to identify the course that work of this kind may take in the future by underlining both the opportunities that it presents and the difficulties that it entails.

Menaker will briefly review the work that has led to the hypothesis that the pineal organ and suprachiasmatic nuclei function as clocks, regulating a variety of expressed circadian rhythms in several classes of vertebrates. He will then summarize what is known of the oscillatory properties of the vertebrate pineal in vitro-its responsiveness to light, temperature and pharmacological agents-and will discuss what this information suggests about the pineal's role in the circadian system of the organism.

Block will describe experiments in which he has delimited the circadian pacemaker in the eye of the mollusc Bulla gouldiana. He has obtained long term intracellular records from photoreceptors and lower retinal neurons of the isolated eye. Photoreceptors fail to exhibit spontaneous changes in resting membrane potential in darkness while lower retinal neurons undergo conspicuous rhythmic change in spike frequency and membrane potential. In addition, surgical removal of the entire photoreceptor region does not prevent the expression of the circadian rhythm or alter its period. Thus, circadian pacemakers reside among the lower retinal neurons, perhaps within individual cells.

Eskin will discuss his experiments demonstrating that some aspects of circadian timing in the isolated eye of Aplysia are regulated by the putative neurotransmitter serotonin. He has used serotonin as a probe with which to approach identification of the mechanisms of entrainment and the involvement of cAMP and protein synthesis in circadian timing.

After reporting his experiments on the biochemical regulation of circadian rhythms of melatonin synthesis in the avian pineal, Takahashi will bring the evidence currently available from both invertebrate and vertebrate in vitro systems to bear on the question of whether there are some common biochemical pathways underlying the generation and control of circadian oscillations.

300 SYMPOSIUM. BIOCHEMISTRY OF SYNAPTIC REGULATION. R.B. Kelly, Univ. of California, San Francisco, and M.B. Kennedy, California Inst. of Tech. (Co-chairs); P.V. DeCamilli, Univ. of Milan; R.J. Lasek, Case Western Reserve Univ.; J.H. Schwartz, Columbia Univ. CPS.

The symposium will focus on molecular events that underlie the control of synaptic transmission. The strength of synaptic connections can be altered by prior activity and by extracellular agents. This occurs by modulation of both transmitter release and postsynaptic excitability. Different synapses can be regulated in different ways. Thus, in order to understand synaptic function, it will be useful to know the molecular machinery responsible for various forms of regulation and the distribution of this machinery in different synapses.

The regulatory second messenger, cyclic AMP, acts primarily, if not exclusively, via two cyclic AMP-dependent protein kinases that are found in most tissues, including brain. Neuronal regulatory systems that involve cyclic AMP-dependent protein phosphorylation have recently been studied by physiological and immunohistochemical techniques. J. Schwartz will describe experiments that demonstrate regulation of transmitter release by cyclic AMP-dependent phosphorylation in synapses of Aplysia sensory neurons. P. DeCamilli will discuss the localization within synapses of cyclic AMP-dependent protein kinase subunits, and two proteins that it phosphorylates, synapsin I and MAP_2.

Regulatory systems that are controlled by increases in the concentration of calcium ion are not as clearly understood, at the biochemical level, as are those controlled by cyclic AMP. In contrast to cyclic AMP, calcium ion regulates a number of different enzymes and proteins that are often specific to particular tissues. Many of these proteins are activated via the small calcium-binding protein, calmodulin. R. Kelly will discuss two newly identified calmodulin-binding sites on the membrane of synaptic vesicles. M. Kennedy will describe a recently purified calcium and calmodulin-dependent protein kinase that is highly concentrated in brain tissue, and is a major component of brain postsynaptic density preparations. R. Lasek will present evidence that calcium-activated proteases carry out essential processing steps in the formation and maintenance of synapses.

The speakers will discuss the implications of their findings for models of synaptic regulation.

GABA AND BENZODIAZEPINES: BINDING SITES II

301.1 DIVERSE GROUPS OF PSYCHOTROPIC DRUGS INTERACT WITH GABA/PICROTOXIN RECEPTOR COMPLEXES. R.F. Squires and E. Saederup, Rockland Research Inst., Orangeburg, NY 10962. 35S-t-butylbicyclophosphorothionate (TBPS) binds with high affinity to brain-specific picrotoxin binding sites. The binding of TBPS is entirely dependent on ions and is, in the presence of Eccles anions, potently inhibited by GABA and all GABA-A receptor agonists in a way which can be reversed by bicuculline, R 5135 and other known competitive GABA antagonists. (Squires et al. Mol. Pharm. 23: 326-336, 1983). The following groups of substances were recently found to be moderately potent inhibitors of TBPS binding: Cannabinoids: Dimethylheptylpyran (IC_{50}=7.5μM), Δ9-THC (11μM), Δ8-THC (14μM),cannabinol (18μM),nabilone (21μM). Steroids:Androsterone(1.4μM), diethylstilbestrol(3.2μM), progesterone (46 μM). Butyrophenones: clofluperol(14μM benperidol (18μM). droperidol (27μM), trifluperidol (84μM). Carbamates: Nisobamate (22μM), CI384 (29μM),carisoprodol (120μM), ethinamate (350μM), meprobamate (ca 1000 μM). Alcohols and phenols: olivetol (49μM), o-hydroxy biphenyl (52μM), α-chloralose(120μM), ethyl octynol (120 μM),CI383 (170 μM),2,2,5-trimethylphenol (200μM),ethchlorvynol (340μM), methylpentynol (7,000μM). Quinazolones:ethaqualone (13μM), methaqualone (43μM). Others:EMD 41717 (10μM), clomethiazole (47μM), M&B 7150 (63μM), pizotifen (90μM), IB 503 (130μM), fenimide (160μM), The inhibitions of all the substances listed above are strongly reversed by the competitive GABA antagonist R5135. New convulsants: neopentylbicyclophosphate (0.13μM), cyclopentylbicyclophosphate (0.16μM), 6-Cl-pentaMethyleneTetrazole (55μM), 6,6-di-Cl-penta MT (25μM), 6,6-di-Cl-hexa MT (13μM),8-sec-butyl-penta MT (35μM), 8-t-butyl-penta MT (3.8μM), hepta MT (140μM),α,α,β,β, tetramethyl succinamide (130μM). The inhibitory effects of these substances were not reversed by R5135. Substances which completely reverse the inhibitory effect of GABA (5μM): Bicuculline (EC50=3.8μM), Brucine (11μM), pipemidic acid (1mM),R5135 (0.0085μM),Strychnine (10μM),d-Tubocurarine (20μM),. Substances which partially reverse the inhibitory effect of GABA (5μM): 4-Benzyl piperidine (Max. reversal 41%, EC50=146μM) Clodazone (29%, 73μM), Clozapine(22%, 23μM), Emetine (65%,148μM), Ro 5-4864 (14%,ca.1μM), Thebaine (50%,91μM). These latter substances may define sub-populations of GABA-A receptors coupled to TBPS binding sites. Benzodiazepines: Clonazepam (1μM) potentiates the inhibitory effect of GABA by about a factor of 2.

301.2 PERMEATION OF [^{3}H]GABA INTO THE RAT BRAIN: THE EFFECTS OF GABA-TRANSAMINASE INHIBITION. A. Krantis (SPON: P.A. WALICKE). Division of Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A 0R6.

It is well established that γ-aminobutyric acid (GABA) does not readily pass the blood-brain barrier, however the nature of this barrier remains unknown. A current hypothesis holds this barrier to be due to the activity of its primary catabolic enzyme, GABA-2-oxoglutarate aminotransferase (GABA-T), localized to the cerebral microvascular endothelial cells (Van Gelder, N.M. J. Neurochem. 12:239-344, 1965).

In the present study the involvement of GABA-T in the blood-brain barrier to GABA was investigated using a sensitive quantitative radiotracer technique modified after Rapoport et al, Brain Res. 150:653-657, 1978, which allows measurement of tracer permeation as a cerebrovascular permeability-area product, PA. PA was calculated from the ratio of parenchymal tracer concentration accumulated during 30 mins after i.v. bolus injection, relative to the area under the curve of arterial plasma tracer concentration vs time (plasma integral). In Sprague Dawley rats (male, 250-300 g), subjected to peripheral GABA-transaminase inhibition (AOAA, 25 mg/kg) and occlusion of hepatic circulation (a primary site of GABA metabolism), permeation of [^{3}H]GABA as measured by PA ($s^{-1} \times 10^{-6}$) was poor, ranging from 11.3 ± .11 to 20.3 ± .15 for the brain regions tested. Chromatographic (TLC) analysis together with assessment of lyophylized plasma samples showed the radioactivity localized to brain parenchyma was derived from the permeation of unchanged [^{3}H]GABA and not its metabolites. Pretreatment with GABA-T inhibitors (AOAA or Gabaculline) using time-dose regimens shown histochemically to also inhibit endothelial GABA-T activity did not cause any increased permeation of [^{3}H]GABA into the brain.

The results of this study afford direct evidence that the blood-brain barrier to GABA in the rat is unrelated to the activity of GABA-T localized to cerebrovascular endothelial cells.

301.3 ANTICONVULSANT ACTIONS OF A NOVEL SERIES OF GABA UPTAKE INHIBITORS
Libby M. Yunger, John J. Lafferty*, Julia A. Rush*, Bruce R. Lester, George I. Moonsammy*, Peter Zarevics* and Paulette E. Setler. Dept. Pharmacol., Smith Kline & French Labs., Philadelphia, PA 19101.

1-(4,4-Diphenyl-3-butenyl)-nipecotic acid (SK&F 89976A) and 1-(4,4-diphenyl-3-butenyl)-guvacine (SK&F 100330A) are potent, selective inhibitors of synaptosomal and glial GABA accumulation which are centrally active after oral administration. In in vitro studies the compounds were equipotent as blockers of GABA accumulation into either crude synaptosomal (P_2) fractions of rat diencephalon-midbrain (IC_{50} = 0.5 μM) or primary cultures of fetal mouse astrocytes (IC_{50} = 0.8 μM). Thus, these compounds were approximately 10-fold more active than the parent amino acids. In both tissue preparations, inhibition was competitive whether the inhibitor was added simultaneously with, or 15 min prior to, the addition of ^{3}H-GABA. SK&F 89976A and SK&F 100330A, at concentrations up to 100 μM, had no effect on the binding of tritiated GABA, muscimol, or diazepam, nor did SK&F 89976A (1 mM) block the activities of either glutamic acid decarboxylase or GABA-transaminase. Since the synaptic actions of GABA are thought to be terminated by its reuptake into neurons and glia, uptake blockers should enhance GABAergic neurotransmission in vivo. The profile of anticonvulsant activity exhibited by these compounds in the rat suggested a GABAergic mechanism of action. SK&F 89976A and SK&F 100330A attenuated the forelimb extensor component of convulsions induced by bicuculline (0.4 mg/kg, i.v.) with ED_{50}s of 11.3 mg/kg, i.p., and 6.4 mg/kg, i.p., respectively, but had no effect on convulsions induced by strychnine (0.5 mg/kg, i.v.) at three times those doses. The compounds were also potent inhibitors of convulsions induced by pentylenetetrazol (25 mg/kg, i.v.) with ED_{50}s of 6.3 mg/kg and 1.8 mg/kg, respectively, after intraperitoneal administration and 8.5 mg/kg and 5.8 mg/kg, respectively, after oral administration. In addition, SK&F 100330A blocked the hindlimb extensor component of maximal electroshock (60 Hz, 100 mA, 3 sec) convulsions with an ED_{50} of 11.4 mg/kg, p.o. In these tests the potency and profile of SK&F 100330A was similar to that of phenytoin. Thus, these studies indicate that SK&F 89976A and SK&F 100330A represent a novel class of centrally-acting compounds which may possess clinically relevant anticonvulsant activity.

301.4 CHARACTERIZATION, PURIFICATION AND IMMUNOCHEMICAL STUDIES OF MULTIPLE FORMS OF RAT BRAIN L-GLUTAMATE DECARBOXYLASE.
L. A. Denner*, Chin-Tarng Lin*, G. X. Song* and Jang-Yen Wu*. (SPON: R. H. Thalmann). Program in Neuroscience and Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

L-Glutamate decarboxylase (GAD) is the rate-limiting enzyme in the biosynthesis of the neurotransmitter GABA. GAD was purified to homogeneity and found to exist in two forms. These forms differed in their affinity for the cofactor pyridoxal-5'-phosphate (PLP). One form was independent of exogenous PLP for activity and exhibited high affinity for PLP, which could not be removed by extensive dialysis. The other form had low affinity for PLP and required exogenous PLP for activity. These two forms were further characterized using pharmacologic, kinetic and physical analyses. Only the high affinity form was sensitive to the carbonyl-trapping agent aminooxyacetic acid. In addition, the high affinity form was labile when treated at 37°C for one hour while the low affinity form was unaffected by this treatment. Finally, the high affinity form exhibited higher mobility then the low affinity form on 5% nondenaturing polyacrylamide gel electrophoresis (PAGE).

In order to further characterize these forms, rat brain GAD was purified to homogeneity in the presence of saturating PLP. Hypotonic high speed supernatants were chromatographed on DEAE. hydroxylapatite and gel filtration. Final purification was achieved on 5% nondenaturing PAGE. Pure GAD was extracted from the gel and used for the production of both polyclonal and monoclonal antibodies. For the latter, standard hybridoma techniques were used. Mice were injected with the pure GAD preparation and their sera screened for antibody using immunodiffusion. Spleens from mice exhibiting antibody to GAD were fused with myeloma cells. Single clones producing antibody to GAD were then injected to mice for the production of Ascites fluid. These antibodies were then characterized using immunodiffusion and ELISA. Both the monoclonal and polyclonal antibodies will be used to further differentiate and understand the roles of multiple forms of GAD in the function of GABA as a neurotransmitter.

301.5 PURIFICATION AND CHARACTERIZATION OF AN ENDOGENOUS BENZODIAZEPINE-LIKE SUBSTANCE.
J.-Y Wu[1], Y.Y.T. Su[2], and W.M. Huang*[1]. Dept. of Cell Biology[1] and Cullen Eye Inst.[2], Baylor College of Med., Houston, TX 77030.

An endogenous benzodiazepine (BDZP)-like substance has been isolated and purified from rat brain. The purification procedure involved the following steps: 1) homogenization of brain in water containing 1 mM AET, 0.2 mM pyridoxal phosphate, 1 mM EDTA and various protease inhibitors, 2) ultrafiltration through PM 10 membrane which has an exclusion limit of 10,000-dalton, 3) column chromatographies on Sephadex G-50 and Bio-Rad P2 gels which has an exclusion limit of 30,000- and 1,800-dalton, respectively, and 4) two times of reverse phase HPLC C18 column chromatography. The overall purification of BDZP-like substances is over 2,000-fold. The molecular weight of the BDZP-like substance appeared to be less than 1800-dalton. The purified BDZP-like substance appeared to be quite stable to heat treatment, e.g., 100°C for 2 hr and to acid treatment, e.g. 2N formic acid. DNAse, RNAse, peptidase, neutral protease, non-specific protease, trypsin and chymotrypsin have no effect on the endogenous BDZP-like substance. The UV spectra covering from 200-350 nm showed a peak at 313 nm. No clear peak was visible at 260 or 280 nm. The ratio of OD at 260 to 280 nm is around 1.3. Amino acid analysis showed a prominent glycine peak. L-Glutamic and L-aspartic acids could also be detected. However, amino acids could account for only 5-10% of the total material in the highly purified BDZP-like substance preparations based on the optical absorbance at 260 or 280 nm. Based on the above properties, it seems unlikely that the endogenous BDZP-like substance is a simple peptide or nucleotide (support by NIH Grant NS 17038 and 13224).

301.6 INHIBITION OF POTASSIUM-INDUCED CALCIUM UPTAKE BY MICROMOLAR BENZODIAZEPINE BINDING IN INTACT SYNAPTOSOMES.
W.C. Taft* and R.J. DeLorenzo (SPON: G.H. Glaser), Dept. of Neurology, Yale Univ. Sch. Med., New Haven, CT 06510

A specific class of benzodiazepine (BZ) binding sites, that are distinct from the previously described nanomolar (nM) BZ receptor, has been identified in rat brain membranes which binds BZs at micromolar (uM) concentrations in a manner which is saturable, displaceable, and stereospecific (Science 216: 1247, 1982). The potency of BZ binding to this uM site correlated with the potency of these drugs to inhibit maximal electric shock-induced seizures, suggesting that these binding sites may play a role in regulating neuronal excitability in brain. Previous studies have demonstrated that diazepam (DZ) inhibited potassium (K^+)-induced calcium (Ca^{2+}) uptake in synaptosomes (Cell Calcium 2: 365, 1981; JPET 220: 29, 1982), and suggested that this effect may account for some of the neuronal stabilizing properties of the BZs. Evidence is presented in this report that the uM BZ binding sites modulate depolarization-dependent Ca^{2+} uptake in intact nerve terminal preparations (synaptosomes).

Diazepam inhibited K^+-induced ^{45}Ca uptake in synaptosomes at uM levels, but produced no inhibition of ^{45}Ca uptake at nM concentrations. BZ inhibition of K^+-induced ^{45}Ca uptake was stereospecific, concentration-dependent, and observed with clonazepam (CNZ), flunitrazepam, medazepam, and chlordiazepoxide. Ro5-4864 was an effective inhibitor of ^{45}Ca uptake, suggesting that this phenomenon is not mediated by nM BZ receptors. Under these conditions K^+-induced ^{45}Ca uptake is inhibited by the Ca^{2+} antagonists verapamil and Mn^{2+}. Further, DZ inhibits both veratridine- and scorpion venom-induced ^{45}Ca uptake. These results indicate that the Ca^{2+} uptake processes inhibited by uM BZs are voltage-sensitive.

The role of the uM BZ receptors in modifying depolarization-dependent Ca^{2+} uptake was further established by employing irreversible binding to uM binding sites using ultraviolet-activated BZ analogues (CNZ). Following irreversible CNZ binding, synaptosomal fractions were washed to remove unbound CNZ and were assayed for K^+-induced Ca^{2+} uptake in comparison to appropriate control conditions. Under conditions in which uM BZ binding sites were maximally labelled by irreversible binding, K^+-induced Ca^{2+} uptake was maximally inhibited. Drug bound synaptosomes showed no significant morphological differences from controls based on electronmicroscopic analysis. [^{3}H]CNZ affinity binding to intact synaptosomes was found predominantly in synaptosome membrane, and intact synaptosomal [^{3}H]CNZ binding patterns were similar to uM BZ receptor binding in isolated membrane preparations. These results indicate that stereospecific, saturable uM BZ "receptors" modulate depolarization-dependent Ca^{2+} uptake.

301.7 BENZODIAZEPINE AND DIETHYLPYROCARBONATE (DEP) INTERACTION WITH HIGH AFFINITY Ca^{++} CHANNELS. S.M. Shreeve* and D.H. Ross. Dept. Pharm., Univ. Tx. Hlth. Sci. Ctr., San Antonio, TX 78284

High affinity Ca^{++} channels have been characterized in brain synaptosomes with respect to K_{Ca}, $[K^+]_o$ and Ca^{++} channel antagonists. K_{Ca} for this channel was found to be 70 μM at 25 mM $[K^+]_o$ when Ca^{++} influx was measured at 5 sec. The influx of Ca^{++} under these conditions fully supports the release of dopamine in striatal tissue. Ca^{++} influx is competitively antagonized by nitrendipine (30 μM) but noncompetitively antagonized by verapamil (200 μM). Hirsch and Kochman (Neurosci. Abst. #158.13, 1982) have reported possible coupling of benzodiazepine receptors with Ca^{++} channels; however, they did not measure actual Ca^{++} influx. In the present report, we have studied the effects of midazolam and DEP on high affinity Ca^{++} channels in cortex synaptosomes. Midazolam (10-500 μM) inhibited high affinity Ca^{++} influx in cortex with an IC_{50} of 40 μM. Neither picrotoxin (100 μM) nor GABA (500 μM) alone or in combination with midazolam produced any significant antagonism of the midazolam effect. Ethanol (87 mM) did not alter the degree of midazolam inhibition and produced no effect alone.

Under appropriate conditions, DEP reacts primarily with histidine residues, resulting in formation of N-carboxyhistidyl derivatives. pH Curves for Ca^{++} influx suggest that a histidine residue may be in close proximity to the Ca^{++} channel. DEP (0.1-10 mM) produced a concentration-dependent inhibition of Ca^{++} influx with an IC_{50} value of 2 mM. Raising $CaCl_2$ concentrations (7.5 mM) in the preincubation media did not protect against the DEP inactivation of Ca^{++} channels. Since DEP is reported to inactivate benzodiazepine receptors (IC_{50} = 1 mM; Burch et al, Mol. Pharm. 23, 1983), our data suggests that benzodiazepines may act at receptor sites which are coupled to the high affinity Ca^{++} channel. Since picrotoxin and GABA were ineffective in altering the benzodiazepine response, the inhibition of Ca^{++} channels reported here is not likely to proceed via the BZP-GABA complex. Our results do suggest that presynaptic receptors for benzodiazepines may be linked to high affinity Ca^{++} channels.

Supported by U.S. Army Research Contract DAMD 17-81-C-1206.

301.8 BENZODIAZEPINE AND MUSCIMOL BINDING SITES IN ADRENAL MEDULLA: RECEPTORS OR DRUG ACCEPTOR SITES? Y. Kataoka*, Y. Gutman*, E. Costa, A. Guidotti (SPON: Susan Stein) Lab. Preclinical Pharmacology, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032

It has been reported that the behavioral effects of anxiolytic benzodiazepines or anxiogenic beta-carbolines are associated with concomitant peripheral effects due to central stimulation of adrenal medullary function. To elucidate whether these peripheral effects are the consequence of a direct action of benzodiazepine ligands on the adrenal medullary cells, we have examined if adrenal chromaffin cells contain receptors for benzodiazepines. Binding studies using ^{3}H-flunitrazepam indicate that this ligand binds to membranes obtained from cow adrenal medulla or from primary cultured cells with a Kd of approximately 10 nM and a Bmax of approximately 80 fmol/mg prot. The binding of ^{3}H-flunitrazepam is displaced (more than 50%) by diazepam, RO 15-1788 and beta-carboline methyl ester. However, diazepam is 10-20 fold more potent than RO 15-1788 or beta-carboline methyl ester (IC_{50} 20 nM). The low affinity of beta-carboline methyl ester for the benzodiazepine binding sites in adrenal medulla was confirmed by direct binding measurement using ^{3}H-beta-carboline methyl ester. ^{3}H-RO 4864, the ligand for the peripheral benzodiazepine binding sites, failed to bind specifically to the adrenal medulla membranes in the concentration range from 0.4 to 200 nM. Hence ^{3}H-flunitrazepam cannot be considered to bind to a typical "peripheral" recognition site. Moreover this binding was increased by 30-40% by the addition of 100 uM GABA to the incubation medium. This increase was similar to that observed in brain. ^{3}H-muscimol also binds with high affinity to bovine adrenal medulla membranes and to membranes obtained from cultured chromaffin cells. The Kd is approximately 2nM and the Bmax around 20 fmol/mg protein. Similarly to the GABA recognition sites of brain the binding of muscimol to medullary membranes was increased following treatment with 0.05% Triton-X 100. We are presently studying the effect of diazepam and muscimol on spontaneous and ACh-evoked release of catecholamines or enkephalin-like peptides from primary culture of adrenal chromaffin cells. Diazepam and muscimol (up to 10^{-6}M concentration) failed to alter the spontaneous and ACh-induced catecholamine release. However, preliminary experiments suggest that diazepam and muscimol in association change the spontaneous release of enkephalin-like peptides.

301.9 CHARACTERIZATION OF BENZODIAZEPINE BINDING SITES IN LUNG. M.Del Zompo, A.Bocchetta*, G.U.Corsini* and G.L. Gessa*. Institutes of Clinical Pharmacology and Pharmacology, University of Cagliari, Via Porcell 4, Cagliari, Italy.

Benzodiazepine binding sites can be demonstrated in several peripheral tissues, including kidney, heart, platelet mast cells as well as in the spinal cord and in cultured cells of various types. However, they have different pharmacological properties from the classical brain benzodiazepine binding sites. The full significance of the presence of these peripheral sites and their localization is not clear. Brief reports of these peripheral sites in lung have also appeared. To facilitate further studies on this, we present here the basic binding parameters of the peripheral sites in rat lung. Crude membrane fractions of lung from male rats were prepared and H Ro 5 - 4864 binding assay was performed. Scatchard analysis resulted in one straight line, suggesting a single class of binding sites. The K in the rat lung was 4.62 ± .09nM and the Bmax was 4669 ± 925 fmol / mg protein. The binding of H Ro 5 - 4864 reached the equilibrium after 15 min. Dissociation of H Ro 5 - 4864 was with T 1/2 = 5.54 ± 0.08 min and the dissociation rate constant was 0.125 min . The binding of H Ro 5 - 4864 to lung membrane was specific, saturable, Gaba indipendent and increased linearly with increasing protein concentration. The effects of benzodiazepines on kidney and lung function are still unclear and need extensive investigation but some of our data may suggest the hypothesis of a coupling between these binding sites and some ion channels.

301.10 BENZODIAZEPINE/ MUSCIMOL INTERACTION: CHANGES OF MUSCIMOL BINDING CHARACTERISTICS TO MOUSE BRAIN STRUCTURES IN VIVO. P. Ferrero*, E. Costa, A. Guidotti (SPON: R.J.S. Schwartzman). Lab. of Preclinical Pharmacology, NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

GABA recognition sites can be modified in vitro by the addition of anxiolytic benzodiazepines. This modification consists of an increase in the B_{max} of ^{3}H-muscimol or ^{3}H-GABA binding without any change in affinity. Anxiogenic compounds chemically related to the β-carbolines inhibit this interaction. It has been suggested that these molecular mechanisms are operative in explaining the role of GABAergic transmission in the anxiolytic and anxiogenic activities of these benzodiazepine ligands. However, extrapolation from in vitro binding to in vivo receptor modulation is difficult because of many artificial conditions that characterize in vitro experiments (e.g., ion concentrations, membrane disruption, and equilibrium conditions).

We have injected mice with a tracer amount of ^{3}H-muscimol (6.8 nmol/kg i.v.; spec. ac. 30 Ci/mmol) and found that the radioactivity concentrates in various brain structures, peaks within 10 min, and then declines to about 50% of its peak value at 40 min. This radioactivity is composed of authentic muscimol and its metabolites. The percentage of authentic muscimol changes with time and is maximally 15-17% of total radioactivity at 10 min. Injection of cold muscimol displaces 30-35% of authentic ^{3}H-muscimol from various brain structures. When tracer amounts of radioactive muscimol (6.8 nmol/kg) and cold muscimol (from 24 nmol to 5 μmol/kg) are injected together, the displacement is dose related. Maximum displacemant is obtained with intravenous injections of 50 to 80 nmol/kg muscimol. THIP can substitute for cold muscimol in this displacement. The density of specific binding sites is highest in striatum, followed by hippocampus, occipital cortex, and cerebellum. In mice receiving diazepam (0.5 mg/kg, i.p.) the extent of binding of tracer doses of ^{3}H-muscimol is decreased by about 20%. However, this bound muscimol could not be displaced by an excess (4.4 μmol/kg) of cold muscimol. Moreover, in diazepam treated mice, the amount of ^{3}H-muscimol bound to various structures did not decline within 30-40 min as it did in saline treated mice. Evidence will be presented suggesting that diazepam stabilizes and modifies the binding of ^{3}H-muscimol in vivo.

301.11 STIMULATION BY BENZODIAZEPINES OF THE PROSTAGLANDIN D_2 RELEASE IN C_6-GLIOMA CELLS. M.D. Majewska*, D.M. Chuang* and E. Costa (SPON: P. Oliver), Lab. Preclinical Pharmacology, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032

GABA and benzodiazepines recognition sites are located in the membranes of cultured C_6-glioma and neuroblastoma NB_{2a}. We have investigated whether these sites are linked with Cl^- channel or with some other transducer, for instance phospholipase A_2. In C_6-glioma but not NB_{2a} cells, prelabeled with ^{14}C-arachidonic acid, a GABA receptor agonist muscimol stimulates the release of radioactive arachidonic acid into the medium. This increased release is associated with a reduction of a small pool of labeled phosphatidylcholine and phosphatidylethanolamine and is prevented by inhibitors of phospholipase A_2 including the Ca^{2+}-chelator EDTA. This process is not related to the GABA uptake system because it is insensitive to the uptake blockers beta-alanine and nipecotic acid. The muscimol-induced release of ^{14}C-arachidonate is blocked by bicuculline but is unaffected by picrotoxin and pentylenetetrazol, indicating that the effect is mediated by GABA recognition sites which are uncoupled from Cl^- channels. The phospholipase A_2 activation by muscimol is potentiated by flunitrazepam, midazolam, diazepam and medazepam (given in their potency order), but not by clonazepam. This benzodiazepine potentiation of muscimol effect is antagonized by RO-5-4864 but not by beta-carbolines, suggesting that a peripheral type of benzodiazepine recognition site is involved. Analyses of the radioactive arachidonate metabolites released in the medium revealed that muscimol alone stimulates the release of free arachidonic acid; however, when muscimol is accompanied by diazepam, a substantial amount of prostaglandin D_2 is released together with the free arachidonic acid. We propose that in glial cells possess a GABA-benzodiazepine receptor linked to mechanisms activating prostaglandin D_2 biosynthesis. A linkage of glial cells to neuronal function via prostaglandin D_2 will be proposed as a working model.

301.12 BENZODIAZEPINE RECEPTOR-MEDIATED INCREASES IN PLASMA ACTH. P.T. Ninan*, H. Schulte*, G.P. Chrousos*, J.N. Crawley, P. Skolnick* and S.M. Paul*. (SPON: J.W. Daly). NIH, Bethesda, MD 20205.

The anxiolytic actions of the benzodiazepines (BZ) have been attributed to the interaction of these compounds with high affinity, stereospecific receptors in the central nervous system. 3-Carboethoxy-β-carboline (β-CCE) has a high affinity for BZ receptors and can antagonize the pharmacological actions of BZ in rodents. We have previously demonstrated that administration of β-CCE to rhesus monkeys elicits a profound behavioral syndrome reminiscent of "anxiety" accompanied by significant elevations in heart rate, blood pressure, plasma catecholamines and cortisol. The antagonism of these effects by both diazepam and the "pure" BZ antagonist Rol5-1788 strongly suggests that β-CCE's actions are mediated through the BZ receptor.

To determine whether the increase in plasma cortisol was the result of specific activation of the hypothalamic-pituitary-adrenal (HPA) axis, we measured plasma ACTH following β-CCE administration to chair adapted, restrained rhesus monkeys. Further, we compared these responses to those observed after an i.v. pharmacologic dose (10 µg/kg) of ovine corticotropin releasing factor (oCRF). Plasma ACTH was measured by a specific and sensitive radioimmunoassay. Within one minute of the β-CCE injection, there was a rapid increase in plasma ACTH which peaked within 20 minutes and returned to baseline over the next 120 minutes. β-CCE (0.1 mg/kg) increased plasma ACTH by 114% while higher doses (0.5 to 1.0 mg/kg) increased plasma ACTH by over 624%. The latter increase is similar to that observed after oCRF (610%). Plasma cortisol increased significantly in response to β-CCE and peaked between 45 and 60 minutes. The time course of the increase in plasma cortisol suggests that the β-CCE induced elevation of plasma cortisol is mediated through ACTH. The magnitude and temporal characteristics of the ACTH and cortisol responses to β-CCE are similar to those observed with maximal doses of oCRF and are similar to those reported with insulin-induced hypoglycemia in man.

Thus β-CCE causes maximal secretion of ACTH in a manner similar to that observed during standardized metabolic stress test in man (Insulin Tolerance Test). The β-CCE model of "anxiety", may also provide a method for studying the relationship between activation of the HPA axis, "anxiety" and metabolic "stress".

301.13 SYNAPTOSOMAL BASIC PROTEINS: DIFFERENCES FROM MYELIN BASIC PROTEINS. F. Vaccarino, E. Costa, A. Guidotti. Lab. Preclinical Pharmacology, NIMH, Saint Elizabeths Hosp., Washington, D.C.20032

The binding of GABA to specific recognition sites on postsynaptic neuronal membranes is regulated by a peptide termed GABA-modulin (GM). GM purified from rat brain and added in vitro to crude synaptic membranes, noncompetitively reduces the number of high affinity GABA recognition sites. Hence, it appears to act as a coupling factor in the reciprocal interaction between recognition sites and other components of GABA/benzodiazepine receptor complex. Moreover, GM can be phosphorylated in vitro by cAMP-dependent protein kinase and this phosphorylation results in a loss of its inhibitory activity (Wise et al., PNAS 80: 886, 1983). In order to be functionally relevant the interaction between GM and GABA/benzodiazepine receptor observed in homogenates should be demonstrated at the synaptic level. To establish whether the location of GM is compatible with its proposed functional role, synaptosomal membranes were purified by the flotation-sedimentation density gradient centrifugation. These membranes contain high concentrations of GABA binding sites (Bmax 5 pmol/mg protein) as well as GM (~10 µg/mg protein). GM is a constituent of these membranes because it cannot be easily extracted by washing with iso-osmotic solutions but it is extracted with detergents. Treatment of synaptosomal membranes with 0.05% Triton X 100 results in a decrease of GM content and in a parallel 2-3 fold increase in ^{3}H-GABA binding. GM extracted and purified from rat brain synaptosomes is composed of 127 amino acid residues, is rich in arginine and lysine residues; therefore the protein is basic. The above characteristics link this peptide to the general class of brain basic peptides. In particular synaptosomal GM is similar in structure to the small molecular weight myelin basic protein (RSBP); however, GM has a different amino acid composition, is richer in GLX (+7) and Lys (+4) residues and contains less Arg (-6) residues. Synaptosomal GM has a slightly different retention time on HPLC reverse phase C18 column using TFA/acetonitrile as eluting buffer. The tryptic map shows differences in four fragments between the two proteins. The migration of synaptosomal GM in SDS-PAGE and urea acidic gels is similar to that of the RSBP: however, the isoelectric focusing gel electrophoresis employing nonequilibrium pH gradient reveals a different chromatographic mobility: GM migrates toward the cathode as three major bands at a rate slower than that of RSBP. These results indicate that GM is an integral constituent of synaptosomal membrane and therefore may play a role in the regulation of GABAergic transmission.

302.1 AXON OUTGROWTH IN CULTURED WING DISCS OF DROSOPHILA. Seth S. Blair* and John Palka. Department of Zoology, University of Washington, Seattle, WA 98195.

During the metamorphosis of Drosophila melanogaster, wings are formed from the wing imaginal discs. The sensory neurons of the wing differentiate and grow during the first few hours of pupariation. By using antibodies against horseradish preoxidase, it has been possible to stain and identify the sensory neurons during this period, and show that they arise in a stereotyped sequence, laying down a characteristic pattern of nerve bundles which project proximally into the CNS.

Other workers have been able to culture isolated fly imaginal discs and, under appropriate conditions, to induce disc eversion and the differentiation of some adult structures. We have cultured everting wing discs in serum-supplemented Schneider's or Robb's medium and shown that neuronal differentiation and outgrowth will take place under such conditions. While the overall shape and size of the developing wing remains somewhat stunted, neuronal differentiation and outgrowth follow the normal developmental sequence and largely normal nerve bundles are formed.

Such cultured discs may also be surgically manipulated in an attempt to uncover the mechanisms underlying the orderly formation of sensory nerves. For example, axon outgrowth may be followed in cut or fragmented wing discs. Preliminary results indicate that, in distal dics fragments cut before or at the time of apparent axon outgrowth, neurons grow proximally along largely normal pathways. Thus proximal cues, such as the location of more proximally located nerve cells, seem not to be necessary at that time for the normal outgrowth of distally located neurons. Further surgical experiments are in progress which bear on the problem of axonal guidance within the developing wing.

302.2 GROWTH OF SENSORY AXONS IN THE DEVELOPING WING OF DROSOPHILA MELANOGASTER. Marjorie A. Murray and John Palka. Department of Zoology, University of Washington, Seattle, WA 98195

Neurons which will innervate sensilla on the wings of Drosophila develop from epithelial cells and begin to differentiate at the very onset of metamorphosis, i.e., just as the wing is beginning to form from its imaginal disc. Using an immunocytochemical stain, we have chronicled the appearance of a small number of identified sensory neurons and the growth of their axons which lay down the stereotyped nerve pathways of longitudinal veins 1 and 3 (L1 and L3).

During the first hour after pupariation (AP) dendrites and cell bodies of five identified neurons which will innervate campaniform sensilla appear. In another one to two hours, these cells initiate nearly simultaneous axon outgrowth, which is oriented toward the wing base. By 6 hrs AP, these axons are 50 to 75 microns long, forming apparently unconnected segments of the adult pathways. They join up shortly thereafter. Only at 12 hrs AP do the closely packed bristle neurons along vein L1 begin axonogenesis, although their dendrites and somata stain as early as 2 hrs AP. By 15 hrs AP the basic adult nerve pattern has been established.

Current hypotheses regarding the establishment of peripheral axon pathways suggest that neuronal cell bodies differentiate along presumptive routes and that axons navigate from one soma to the next by filopodial exploration. Our observations on Drosophila wings suggest that factors other than neuron-neuron contact may also be operating. First, two of the early neurons begin growing their axons toward yet undifferentiated neuronal targets. Second, in another striking instance, two axons are always seen to grow simultaneously toward a meeting place some distance from their cell bodies, although the simplest prediction from a "steppingstone" hypothesis would be for the axon of one neuron to grow directly to the soma of the other. Such axonal behaviors suggest that growth cones may follow cues on epithelial cells or extracellular material as well as those on other neurons.

Axons may use not only molecular cues but may also be guided by physical landmarks in tissue through which they grow. We have found a surprising degree of organization in the early wing, where one micron plastic cross sections reveal the presence of veins as early as 4 hrs AP. An analysis of initial axon outgrowth in relation to vein walls and their epithelial surfaces is underway.

Supported by grants NS07778 from the NIH and 8204088 from the NSF.

302.3 FORMATION OF NEURONAL PATHWAYS IN IMAGINAL DISCS OF DROSOPHILA. Yuh Nung Jan[1], Alain Ghysen*[2] and Lily Yeh Jan[1]. [1]Department of Physiology, Univ. of California, San Francisco, CA 94143, [2]Lab. de Génétique, Univ. Libre de Bruxelles, Belgium.

Axon outgrowth and pathway formation has been extensively studied in many organisms. These studies suggest that chemical cues on the surface of neurons and paths may play important roles in this process, although the nature of such chemical cues is largely unknown. One approach to this question in molecular terms is to take advantage of the well studied genetics and molecular biology in Drosophila, and to identify and isolate genes that are important for the formation of neuronal pathways.

Thus far a major problem to this approach is the small size of early neurons in Drosophila, which makes it difficult to analyze the behavior of single neurons during the initial process of pathway formation. Using neuronal specific monoclonal antibodies we have searched the nervous system in Drosophila for a preparation accessible to experimentation. Here we report that the formation of neurons and pathways in the imaginal discs can be analyzed at single cell level with the help of a monoclonal antibody. In the leg disc, six neurons can be seen with their axons joining the larval nerve early in the third instar larval stage. This pattern remains constant during third instar larval development. Within the first two hours of pupariation the leg disc evaginates, bringing these neurons to the tip of the future leg and thus forming the anterior medial and the posterior lateral neuronal pathways. At the same time new sensory neurons start to appear at characteristic positions with precise timing and send their axons to join one of the two pathways. In contrast to the leg disc, no larval neurons which could serve as guidance are present in wing discs; the first neurons appear a few hours after puparium formation. (Detailed analysis of neuronal pathways in older wing discs have been previously described by Palka et al., Nature 294, 447, 1981).

Formation of neuronal pathways in imaginal discs is amenable to detailed studies: The early events take place in the first few hours of pupariation, during which period imaginal discs can be explanted and still evaginate and develop in culture media. The number of neurons that appear in this early period is small and the size of these neurons (8-15μ) is large enough for impalement, making it possible to study mutations affecting pathway formation at the cellular level. Preliminary studies have already revealed an interesting mutant in which the six neurons in the leg disc appear normal during larval development. However, they degenerate shortly after puparium formation and no new sensory neurons appear. The simplicity and accessibility of this preparation may eventually be used in identifying genes important in pathway formation.

302.4 GUIDANCE FEATURES FOR NAVIGATION BY PERIPHERAL PIONEER NEURONS

Michael Caudy and David Bentley. Biophysics Group and Department of Zoology, University of California, Berkeley, CA 94720

In limb buds of grasshoppers, the first pair of pioneers (Ti1) project their axons along a non-adjacent chain of immature neurons, termed guidepost cells. We have tested the hypothesis that the placement of these cells is a dominant feature of the guidance mechanism: in young embryos, the most proximal guidepost cells (CT1) were identified by direct labeling with fluorescein-tagged neuron-selective antibodies. The cells were killed with a UV microbeam, and the embryos were then cultured until the Ti1 growth cones should have reached the CNS. Experimental limbs, culture-control limbs, and irradiation-control limbs were re-stained with an indirect-labeling procedure, using rhodamine-tagged antibodies. In limbs where the guidepost cells were killed, pioneer growth cones failed to navigate their normal route, showed abnormal branching, and often failed to contact the CNS. We conclude that the guidepost cells are an essential part of the normal guidance mechanism.

We have also observed the morphology of about 3000 pioneer neurons fixed in various stages of navigation to the CNS. Phenomana observed in this material suggest that additional mechanisms constrain the routes taken by pioneering growth cones: (1) Ti1 growth cones are disposed essentially two-dimensionally on the inner surface of the limb epithelium. This behavior, which could be due to a preferential adhesion to epithelial cells or to the basement lamina, greatly constrains the possible routing. (2) Ti1 growth cones almost always emerge from the proximal surface of the cells; this position is at the apparent pole of the cell, and might reflect an influence of internal (possibly cytoskeletal) organization. (3) After emerging from the cell body, Ti1 growth cones almost always extend proximally for several cell diameters before they appear to contact the most distal guidepost cell. During this portion of the route, they take a somewhat meandering and varied path, with a great diversity of growth cone configurations. Rarely, growth cones grow in the wrong direction, and in the longest (metathoracic) limb may even project distally. These observations suggest a weak, proximally orienting cue (which could be diffusible or could be a gradient of adhesiveness). (4) Upon reaching the vicinity of guidepost cells, Ti1 growth cones may extend exceptionally long filopodia directly toward the guidepost cells, or filopodia whose bases are oriented in a different direction may curve toward the guidepost cell. These filopodia are not in contact with the guidepost cells or with their filopodia. This suggests a non-contact cue to the presence and location of guidepost cells. (5) Near the proximal border of the femural leg segment, where guidepost cells CT1 and F2 are located, both F2 and Ti1 axons cross or turn sharply along a very straight circumferential route. After traversing a short arc around the perimeter of the limb, they turn off this route and grow toward the CT1 guidepost cells. In experimental limbs where the CT1 cells have been killed, these cells still send major branches along the circumferential route, and axons of another cell, F3, may follow the route for about 150 degrees of arc. These observations suggest the presence of another guidance cue (again, weaker than the effect of guidepost cells); this cue could be related to the intersegmental boundary.

302.5 NEURONAL GROWTH CONES: SPECIFIC INTERACTIONS MEDIATED BY FILOPODIAL INSERTION AND INDUCTION OF COATED VESICLES. Michael J. Bastiani and Corey S. Goodman. Dept. of Biol. Sci., Stanford University, Stanford, CA. 94305

We are interested in the factors that guide individual neuronal growth cones during embryonic development. Here we report on the discovery of a novel and highly specific interaction between identified growth cones in the grasshopper embryo. The first growth cones in the grasshopper CNS, from the MP1, dMP2, and vMP2 neurons (Bate and Grunewald, JEEM, 1981), make divergent and stereotyped choices when confronted with the same environment: the MP1 and dMP2 turn posterior while the vMP2 turns anterior (Taghert et al, DB, 1982). Our previous results suggest that the MP1 filopodia selectively adhere to the pCC neuron as the MP1 growth cone turns posterior; we proposed that this neuron was a "landmark cell" for the MP1 growth cone.

Here we report on TEM serial section reconstructions of the MP1 growth cone and its filopodia at a stage just after its growth cone turns posterior; by this time, the pCC has just initiated its own growth cone. 60 microns of serial thin sections were taken from the anterior edge of MP1 cell body to the posterior edge of the pCC cell body in the T2 segment. The cell bodies and axons of MP1, dMP2, vMP2, and pCC were identified in thin section by their characteristic positions and shapes.

The MP1 growth cone had 28 filopodia extending from its leading 4 microns; all 28 were reconstructed. Some of these filopodia contacted the dorsal basement membrane, the end feet of epidermal cells, the surfaces of unidentified cells, and many unidentified filopodia. However, the majority of filopodia from MP1 (19/28) contacted the pCC. Six filopodia came into intermitent and short (less than 1 micron) contact with the pCC cell body, growth cone, or filopodia. Six others contacted the pCC at its cell body and stayed in close contact for several microns. The remaining 7 filopodia from MP1 were the most interesting. They all first contacted the pCC at its growth cone and were found inserted anywhere from 0.1 to 7 microns into the cell. Coated pits and vesicles typically were present in the membrane of the pCC at the filopodial tips of these insertions. Other filopodia from MP1 that contacted the cell body of the pCC, although closely adherent, did not penetrate into the cell.

The interaction between the MP1 filopodia and the pCC growth cone is remarkably specific; filopodia from other nearby growth cones contact the pCC growth cone, but neither penetrate it nor induce coated vesicles. We are presently ablating the pCC and/or MP1 to examine the role of this specific interaction. This novel interaction of filopodial insertion and induction of coated vesicles occurs in other identified growth cones throughout grasshopper development. Interestingly, similar profiles have been observed in the growth cones of developing amphibians, although the origin and specificity of the insertions is not yet known (Nordlander and Singer, JCN, 1978). Thus, this phenomenon may be a general mechanism mediating specific inductive interactions during development.

Supported by an NIH postdoctoral fellowship to M.J.B., and an NSF grant and a McKnight Foundation Scholars Award to C.S.G.

302.6 NEURONAL GROWTH CONES IN THE ANTENNAE OF THE GRASSHOPPER EMBRYO: GUIDANCE BY EPITHELIAL GRADIENTS AND ADHESIVE HIERARCHIES. John Berlot*, Michael J. Bastiani, Corey S. Goodman. Dept. of Biol. Sci., Stanford University, Stanford, CA 94305

We are interested in the factors that guide neuronal growth cones during embryonic development. We are studying the growth cones of the one the simplest systems in the grasshopper embryo: the ventral (VP) and dorsal (DP) pairs of pioneer neurons in the antennae. Our results suggest that these growth cones are guided by an epithelial adhesive gradient for polarity and neuronal landmark cells for specific turns away from this axial polarity.

About the time the pairs of VP and DP neurons appear at the distal tip of the antenna, a single base pioneer (BP) neuron appears on the ventral surface at the antennal base and extends its growth cone towards the CNS, contacting and fasciculating with a motoneuron growth cone extending outward from the CNS. The VPs are about 100 um from the BP and on the same (ventral) surface of the epithelium when they initiate their growth cones; the DPs are on the opposite (dorsal) surface and over 200 um (and thus beyond the 50 um filopodial grasp) from the BP when they initiate their growth cones. Only when the DP growth cones are within about 50 um of the lateral motoneuron growth cone do they change their axial pathway as they fasciculate with the motoneuron axon and follow it ventrally and medially into the CNS. By this time, the BP is dead.

When the antenna is removed from the embryo and grown alone without the CNS in tissue culture, the DP growth cones extend proximally towards the base of the antennae, eliminating the notion of a chemotactic gradient diffusing from the CNS. They extend along the inside surface of the columnar epithelium with no special landmark cells within filopodial grasp. The only source of initial guidance for the DPs is the epithelium. TEM reveals that the DP growth cones extend under the epithelial basement membrane and their filopodia are in direct contact with the surface of the epithelial cells. These results suggest that the DP filopodia are responding to an adhesive gradient along the epithelial surface. The epithlial cells themselves express an intrinsic polarity in their shape.

Normally, the BP growth cone extends medially towards the CNS as it contacts the motoneuron from the CNS. However, when the antenna is grown alone without the CNS in culture, the BP growth cone extends distally as it contacts the VP growth cones. This polarity reversal is best explained by considering the spatiotemporal relationship of which neuronal surface it first contacts in ovo vs. in vitro. These and other experiments suggest that the filopodia of the pioneer growth cones express an adhesive hierarchy, whereby, when available, neuronal surfaces are prefered over the surfaces of epithelial cells. Given only the epithelium, the growth cones extend proximally along its surface, appearing to follow an adhesive gradient. Given a choice, however, of neurons vs. epithelium, the filopodia preferentially adhere to the neuronal surfaces.

(Supported by grants from NSF and the McKnight Foundation.)

302.7 GUIDANCE OF A GROWTH CONE TO ITS CONTRALATERAL PATHWAY IN THE ABSENCE OF THE AXON IT NORMALLY FOLLOWS DURING GRASSHOPPER EMBRYOGENSIS. Andrew L. Harris and Corey S. Goodman. Dept. of Biol. Sci., Stanford University, Stanford, CA 94305

How do the first growth cones pioneer commissural pathways? Is there a change in their substrate preference after they cross the midline? Here we report on experiments in which we remove the axon normally followed by an identified growth cone.

In each embryonic segment of the grasshopper CNS, one axon bundle in the posterior commissure is pioneered by neuron Q1 and its contralateral homologue, and a slightly more anterior bundle by neuron L1 and its homologue. Both cells lie laterally and extend their growth cones nearly simultaneously toward the midline along the dorsal basement membrane (DBM) within a few microns of each other, but the axons do not fasciculate. Near the midline, the filopodia of both growth cones contact those of both contralateral homologues, yet each becomes dye-coupled only to its homologue. The growth cones drop off the DBM and grow laterally along the homologue's axon. At the contralateral side, Q1 turns posterior along the MP1/dMP2 pathway. Shortly thereafter, Q1 and its sibling Q2 die.

33% grasshopper embryos were grown outside the egg case in a modified RPMI medium supplemented with juvenile hormone and beta-ecdysone (BE). At low levels of BE, Q1 and Q2 developed normally. At higher levels, Q1 and Q2 died while development of other neurons appeared normal. This suggests that in ovo the deaths of Q1 and Q2 is triggered by the normal rise of BE.

We ablated Q1 before it reached the midline and maintained the embryo in culture to allow the remaining Q1 to develop further. Q1 was identified and staged by filling it with Lucifer Yellow (LY) under Nomarksi optics, and was killed by application of large currents and/or mechanical disruption with the microelectrode. Following growth in culture, the remaining Q1 was filled with LY, further visualized with the anti-LY antibody, and other axons visualized with the I-5 monoclonal antibody.

In the absence of its contralateral homologue, Q1 reached the midline along the DBM but then followed an aberrant path. At the midline it turned several microns anterior and then grew laterally along the contralateral L1 axon, an axon it normally contacts but ignores. At the contralateral side, Q1 left the L1 axon to run posterior along the MP1/dMP2 pathway. Thus, in the absence of the axon along which it normally grows past the midline, Q1 grows along another axon in the same commissure.

It is intriguing to note that the axon of L1 is well within filopodial grasp of Q1 for virtually its entire length, yet Q1 does not become associated with L1 or any other axon until after reaching the midline. Prior to reaching the midline, it prefers to grow along the basement membrane, establishing dye-coupling contacts via its filopodia with specific somata. Only after crossing the midline does it prefer to grow along the axon of another cell (normally its homologue, but in this case, L1's). This result may indicate that the substrate preference and resultant pathway choices of this growth cone changes once it reaches the midline of the developing ganglion.

(Supported by an NSF grant and McKnight Scholars Award.)

302.8 ABLATION OF A SPECIFIC AXONAL PATHWAY RETARDS THE EXTENSION OF AN IDENTIFIED GROWTH CONE IN THE CNS OF THE GRASSHOPPER EMBRYO. Jonathan A. Raper, Michael Bastiani, and Corey S. Goodman. Dept. of Biol. Sci., Stanford University, Stanford, CA. 94305.

We have previously described the development of the G neuron in the second thoracic ganglion of the grasshopper embryo. (Raper, Bastiani, and Goodman, 1983a,b, J. Neurosci. 3:20,31). G's primary growth cone crosses the ganglionic midline, and advances laterally to a reproducible location in the neuropil. There it turns anteriorly, fasciculating upon one identified bundle of axons within the embryonic axon scaffold. This fascicle is pioneered by the axons of the A1, A2, P1, P2, and the (recently identified) P3 neurons.

In 5 semi-serial EM reconstructions of the A/P fascicle, the tip of G's growth cone was found to contact one or more of the P axons in 4 cases, one or both of the A axons in 0 cases, and no axon in 1 case. G's filopodia contacted the A/P fascicle in preference to other fascicles, but showed no all-or-none preference for P over A.

We have now assayed the effect upon G's morphology of deleting the A1,2 axons, the P1,2,3 neurons, or the A1,2 axons and P1,2,3 neurons. Our results support the hypothesis that growth cones can recognize and extend upon specific, differentially labelled axons.

The A1,2 cell bodies are in T3. Their axons were prevented from reaching the T2 neuropil by cutting the lateral portion of the ganglionic connective. The P1,2,3 cell bodies were mechanically killed with a microelectrode. Manipulations were performed on one side only so that the other side served as a control. Embryos were cultured for 2 days on membrane carriers at the air-water interface with a RPMI based media at 28°C and 5% CO_2.

Deletion of the A1,2 axons together with the P1,2,3 neurons prevents G's anterior extension past the center of the ganglion. The same effect is achieved by deleting only the P1,2,3 neurons even though the A1,2 axons course through the ganglion. Deleting only the A1,2 axons has no effect upon G's behavior. None of these manipulations prevent G's growth cone from halting its lateral extension at approximately the normal location, and extending in the posterior-anterior axis.

These results suggest that: (1) the A/P fascicle plays an important role in guiding G anteriorly through the neuropil, (2) G's growth cone is able to distinguish between the P and A axons, and (3) the A and P axons alone do not instruct G to halt its lateral extension and turn onto the posterior-anterior axis.

(Supported by grants from the March of Dimes and NSF)

302.9 TRANSIENT EXPRESSION OF CELL SURFACE ANTIGEN ON TWO NEURONS THAT SHARE A COMMON FINAL PATHWAY AND TARGET IN THE GRASSHOPPER EMBRYO. K.J. Kotrla* and C.S. Goodman (spon: R. Akers). Dept. Biol. Sci., Stanford University, Stanford, CA 94305.

In the grasshopper embryo, the filopodia of identified growth cones contact the surfaces of many axon bundles, yet by their selective adhesion appear to guide the growth cones along particular pathways (see Raper, Bastiani, and Goodman, this volume). These results suggest the 'labeled pathways' hypothesis whereby the axonal pathways are differentially labeled, and growth cones are determined to make specific pathway choices using these labels. We are interested in the molecular nature and genomic organization of these axon surface labels that in part encode neuronal diversity and specificity. As a first step, we made monoclonal antibodies (mabs) in search of cell surface antigens on specific subsets of embryonic axons.

5 mabs recognize cell surface antigens on different, small subsets of axon bundles early in development. For example, the mes-3 mab recognizes a surface antigen on the axons in the medial longitudinal pathway pioneered by the MP1 and dMP2 neurons; other bundles in the 40% axonal scaffold are not stained. Thus, axonal pathways are antigenically distinct while growth cones are choosing amongst them.

We have focused our attention on the mes-2 mab. With Lily and Yuh Nung Jan, we immunized mice first with whole Drosophila embryos and boosted with 40% grasshopper embryo nerve cord in the hope of enriching for mabs that can be used at the cellular level in the grasshopper and at the molecular level in Drosophila.

In the grasshopper, mes-2 stains a cell surface antigen present on a specific subset of embryonic neurons. In the T3 segment at 50%, the mes-2 antigen is expressed on only 3 of the 1000 neurons per hemiganglion. Two of the staining cells are the identified motoneurons FETi and SETi, whose growth cones follow the same final pathway and innervate the same (ETi) muscle. The third neuron is a previously unidentified interneuron.

Although these two motoneurons (FETi and SETi) arise from different neuroblasts, they alone amongst their two families share a final pathway and target. We hypothesize that the mes-2 antigen may be involved in either their pathway or target recognition. This notion is supported by the temporal pattern of expression of the mes-2 antigen. The antigen is not expressed while their two growth cones extend in different central and peripheral pathways. Expression begins when the FETi growth cone reaches the SETi axon and follows it to the muscle. Their expression of the mes-2 antigen is transient during this period of axonal outgrowth; after reaching and innervating their common muscle, expression of the mes-2 antigen dramatically decreases. We are currently testing the hypothesis that this antigen is involved in their final pathway and/or target selection.

Fortunately, the mes-2 antigen appears to be expressed on a subset of neurons in Drosophila. Thus it may be possible to use the molecular genetics of the fly to isolate the molecule and investigate its role during embryonic development.

Supported by NIH grant and McKnight Foundation Scholars Award.

302.10 THE ACCURATE INITIAL OUTGROWTH OF SYMPATHETIC PREGANGLIONIC AXONS IN THE EMBRYONIC RAT. Eric Rubin, Dept. of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO 63110.

In adult mammals, the innervation of the sympathetic superior cervical ganglion (SCG) arises from a well-defined set of thoracic spinal segments (Njå and Purves, 1977). Furthermore, in each of these segments a characteristic number of preganglionic cells project to the SCG (Rubin and Purves, 1980). The developmental events that determine which cells will send axons to the ganglion are not understood. To examine this problem, I have studied the growth of preganglionic fibers to the SCG in the embryonic rat.

Silver staining and orthograde transport of horseradish peroxidase (HRP) from the spinal cord indicate that few preganglionic fibers have reached the rat sympathetic chain on day 12 of gestation (E12; day of conception=E0). By E14, a substantial projection has developed, and HRP retrogradely transported from the SCG labels one-third the adult number of preganglionic cells. Cells labeled at this age are found only in the segments that innervate the SCG in maturity; moreover, their numbers already show the adult distribution across these segments. The axons of retrogradely labeled preganglionic cells are visible, and on E14, as in the adult, these fibers leave the spinal cord only through the ventral root at the level of their cell bodies (Rubin and Purves, 1980).

The accuracy of the early projection to the SCG does not appear to result from mechanical constraints placed on growing preganglionic fibers. On the contrary, HRP-labeled preganglionic axons on E14 show no tendency to fasciculate selectively. They disperse across the full diameter of the sympathetic trunk, and labeled fibers that run together for long distances in the trunk may diverge to follow widely separated, often indirect routes to the SCG.

Thus, the mature pattern of projection from the spinal cord to the SCG is apparently established by the accurate initial outgrowth of the preganglionic axons. Axon guidance in the sympathetic system appears to operate at the level of single fibers, and to take account of the segmental level of origin of each axon.

Njå and Purves (1977). J. Physiol. 264: 565.
Rubin and Purves (1980). J. Comp. Neurol. 192: 163.

Supported by grants from MDA and NIH (NS11699) to D. Purves.

302.11 STEREOTYPED AND VARIABLE GROWTH OF REDIRECTED MAUTHNER AXONS. M.J. Katz. Dev. Genetics & Anatomy, Case Western Res., Cleve. OH 44106.

To assay the guidance cues that organize the projection tracts of the vertebrate CNS, individual identifiable axons were redirected in amphibian embryos. Mauthner axons (M axons) were redirected by grafting supernumerary hindbrains in Xenopus embryos. The 63 experimental M axons thus produced included donor axons growing into the host CNS and host axons that grew through the graft or that were redirected in the host CNS. Two major phenomena were observed. As previouly reported, caudal to the optic chiasm the experimental M axons grew along a single ipsilateral stereotyped route -- the basal substrate pathway -- extending in the ventral and ventrolateral marginal zone from the diencephalon to the caudal spinal cord. Rostral to the optic chiasm, the experimental M axons followed variable ipsilateral and contralateral routes. Even pairs of experimental M axons entering the optic chiasm side-by-side eventually grew along different routes in normal forebrains. The contrasting behaviors of M axons growing in the forebrain and of M axons growing elsewhere indicate a fundamental difference in the axon guidance cues between these two regions of the CNS. Moreover, both the stereotyped growth routes of M axons caudal to the optic chiasm and the variable routes rostral to the chiasm are consistent with axon-specific guidance cues. The rationale for this conclusion is as follows: The initial entry of experimental M axons into the histologically normal CNS was an uncontrolled variable. Thus, it is likely that a significant number of the 63 redirected M axons entered normal CNS at stages that were different from normal M axons and that a significant number also grew through areas where no M axon normally grows. In the face of the variable temporal and spatial alignments of M axon with CNS tissue, the M axons evinced a strikingly stereotyped behavior in spinal cord, hindbrain, midbrain, and caudal diencephalon. This suggests that precise spatial or temporal alignments are not absolutely necessary to produce the stereotyped axon growth characteristic of many developing projection tracts. In turn, this is consistent with the idea that the underlying axon growth cues are stable and highly axon-specific (chemospecific). In contrast to their growth more caudally, M axons growing through the forebrain were not strongly constrained to any one route. Even pairs of M axons entering these areas side-by-side eventually grew along different routes -- i.e., the spatial alignment of the axons was insufficient to produce a stereotyped growth route. Normally, a variety of other axon tracts grow through these regions in a stereotyped fashion. The observation that one class of axons is "blind" to the forces that effectively organize another class of axons can be most simply explained by the hypothesis that the organizing forces are axon-specific (chemospecific).

302.12 **DEVELOPMENT OF AXONS IN VERTEBRATE NEUROGENESIS STUDIED WITH MONOCLONAL ANTIBODIES**

Susan Hockfield and Ron McKay, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724

In order to examine neuronal diversity in the vertebrate CNS we have generated monoclonal antibodies to the unfractionated adult spinal cord (McKay and Hockfield, PNAS 79, 6747, 1982). We have studied the developmental appearance of several of these antibodies and found that in most cases the antigens are first present post-natally. To obtain markers for earlier events in neuronal differentiation we have now generated monoclonal antibodies to embryonic day 15 (E15) rat spinal cord. Here we report on the developmental appearance of antigens present in axons.

Two classes of monoclonal antibodies among those generated against adult spinal cord gray matter were those that recognize: 1) axons only; and 2) axons and cell bodies. Several antibodies in these classes bind to proteins that comigrate with the 210Kd neurofilament protein on immunoblots of SDS-polyacrylamide gels. For example, antibodies Cat-101 (axons only) and Cat-201 (axons and cell bodies) recognize a 210Kd species and both have been shown by immunoelectron microscopy to bind to internal antigens in axons. Each of these antibodies has a different light level histological staining pattern and shows a different time course of appearance during development. These observations suggest that the 210Kd neurofilament protein may be a heterogeneous group of molecules.

Monoclonal antibodies rat 6-2F2 and rat 6-8G2 were generated against E15 rat spinal cord and recognize axons in the embryonic nervous system. Antibody-stained processes are first seen in E11 rat embryos (neural tube closing begins to occur at E10). The earliest processes arise from neurons in the ventral quadrants of the spinal cord and from peripheral structures (ganglia). Later in development, antibody-positive processes enter the dorsal quadrants of the spinal cord and form small antibody-intense arbors in the developing dorsal horn. Antibody staining of processes at the developing muscle masses closely follows ventral root staining and staining of processes at the skin is correlated with staining of processes in the dorsal horn. Electron microscopic immunocytochemistry has shown that the antigen is present in the tips of growing axons but is not present in the filopodia arising from them. These antigens are present in axons throughout development and in the adult nervous system. The antibodies recognize bands on immunoblots of SDS-polyacrylamide gels, bands that do not comigrate with the major neurofilament species. These antibodies can be used to study the developmental sequence of axon-associated antigens and to follow the development of axon pathways from early stages of axon outgrowth.

303.1 FUNCTIONAL ORGANIZATION ACROSS SINGLE LAYERS OF THE LATERAL GENICULATE NUCLEUS IN THE CAT. Douglas B. Bowling and W. Michael Schoel*, Departments of Medical Physiology and Anatomy, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 1N4

Most of what is known about the physiology and anatomy of the cat's lateral geniculate nucleus suggests that layers A and A_1 are functionally homogeneous across their depths. The layers have been thought of as containing a mix of cell types that are about equally distributed across each layer. Thus the way in which visual information is processed in the A layers has not been thought of as being different at different depths.

Recently however, results from several studies have suggested that the layers may be functionally differentiated across their depths. Mitzdorf and Singer (1977) analyzed electrically evoked potentials in the geniculate and concluded that X and Y inputs are at least partially segregated between the upper and lower parts of the A layers. A second observation, recently made by Movshon (1981) is that Y cells near the tops of the A layers respond to higher spatial frequencies than Y cells near the bottoms of the layers. Both of these physiological results can be interpreted in terms of the geometry of the afferent terminal distributions which have recently been revealed by intra-axonal injections of horseradish peroxidase (Bowling and Michael, 1980; Sur and Sherman, 1982). These anatomical studies support the physiological results by demonstrating the relative segregation of X and Y terminal boutons across the layers as well as differences in the lateral distributions of the Y terminals at the tops and bottoms of the layers. The geometry of the afferents suggests that at any given depth in the layer there is probably a unique pattern of relative strengths and lateral effects from afferents that are signalling different kinds of information.

We have been interested in looking further at the question of functional differences with depth in the A layers. In anesthetized cats we have used two experimental approaches to the problem. First, we have looked at visual evoked potentials as a function of depth through the layers. Results from these experiments, which were carried out using full field stimuli, suggest a partial segregation of on and off activity across the layers. This interpretation is based on an analysis of the depths of the sources and sinks that are responsible for the on and off phases of the evoked potential. We are also carrying out single unit studies involving tangengial microelectrode penetrations. This approach allows us to accurately define a cell's depth in the layer and to sample a larger number of cells at a given depth. This work is supported by a grant from the Alberta Heritage Foundation for Medical Research and by MRC (Canada) Grant MA7612.

303.2 QUANTITATIVE ULTRASTRUCTURAL MORPHOLOGY OF IDENTIFIED X- AND Y-CELLS IN THE CAT'S DORSAL LATERAL GENICULATE NUCLEUS. James R. Wilson[1], Susan Van Horn*, Michael J. Friedlander[2], and S. Murray Sherman. Department of Neurobiology and Behavior, SUNY at Stony Brook, NY, 11794.

Two physiologically-identified neurons (one X-cell and one Y-cell) in the A laminae were quantitatively studied with the electron microscope. These are in addition to two neurons which were previously studied qualitatively. Representative samples from about 10% of each neuron's entire dendritic arbors were sampled to obtain an estimate of the types and distributions of synapses contacting them. The estimated total number of synapses onto these cells was approximately 4000 for the X-cell and 5000 for the Y-cell. Nearly all of the retinal terminals were located within 100 um of the center of the soma. Although the presumed cortical terminals (RSD type) were the predominant type (percentage-wise) on the distal dendrites, they were, nevertheless, most concentrated (numerically) between 50-100 um from the soma. F terminals were the most common terminal near the soma, and were particularly numerous near the retinal terminals. The average number of synapses of any type per micron of dendritic length was about 1.0 for both cells.

The major ultrastructural differences observed for the X- versus Y-cells were almost entirely related to the retinal afferents. First, the X-cells had retinal synapses mostly onto dendritic appendages (spines, etc.), whereas Y-cells had most of their retinal synapses onto dendritic shafts near primary branch points. Second, the retinal terminals which contacted X-cell dendrites usually formed triadic arrangements, but Y-cells rarely did so. Finally, the main type of F terminals associated with the X-cells were morphologically different from most of those associated with the Y-cells, and this also related directly to the triadic arrangements; that is, F terminals in the triadic arrangements had distinct morphological features. The presence of triadic arrangements on X-cells correlated with electrophysiological data showing a better signal-to-noise ratio occurring from retina to the lateral geniculate nucleus for X-cells compared to Y-cells. Thus, these morphological differences between X- and Y-cells indicate that they might be the synaptic basis for some of the differential processing of information occurring for the two cell types in the lateral geniculate nucleus. (This research supported by USPHS grant EY03604 and RR-00165)

[1]Present address: Yerkes Primate Center, Emory University, Atlanta, GA 30322. [2]Present address: Dept. of Physiology, University of Alabama, Birmingham, AL 35294.

303.3 MORPHOLOGICAL AND FUNCTIONAL ANALYSIS OF SINGLE NEURONS IN THE MEDIAL INTERLAMINAR NUCLEUS (MIN) OF THE CAT'S LATERAL GENICULATE NUCLEUS. D. Raczkowski and S.M. Sherman, Dept. of Neurobiology and Behavior, SUNY at Stony Brook, NY 11794.

The MIN, like the laminated region of the lateral geniculate nucleus, lam LGN, receives direct retinal input and projects to multiple visual cortical areas. We examined the morphology of MIN relay cells following injections of horseradish peroxidase (HRP) into the optic radiations. Three types of neurons could be identified. The type most frequently observed throughout the MIN shares morphological features of class I and V lam LGN neurons. Type A cells are found throughout the MIN. Their somata are oval or elongated and intermediate in size. 3-10 proximal dendrites issue from the soma at irregular intervals. These dendrites are typically smooth, possess occasional stalked appendages, and are often varicose. The dendritic geometries of these cells are variable; some have radial symmetry, while others are bipolar. Type B cells are generally found in the medial part of the MIN, are characterized by flattened or fusiform somata and bear 2-3 primary dendrites in a bipolar configuration. These dendrites are smooth and exhibit few varicosities. Type C cells are seen rarely and only near the lateral edge of the MIN, and they possess dendrites with clusters of grape-like appendages near dendritic branch points. We have attempted to relate W-, X-, and Y-cell physiological classes to these morphological cell classes. Using micropipettes filled with HRP, we recorded from individual MIN cells and classified them physiologically using a battery of criteria including latency to optic chiasm stimulation, responses to counterphased sine-wave gratings, etc. These neurons were impaled and injected with HRP for subsequent analysis of the soma, dendrites and axon. Virtually all neurons recorded in the MIN were Y-cells, with only a rare W- or X-cell encountered near the lateral border of the MIN. Every successfully injected and recovered MIN neuron in our sample is a Y-cell. Many of these neurons exhibit a type A morphology. Somata of these cells range from 135 to 385μm^2. Dendrites of these cells are smooth and varicose. Occasionally, a dendrite was observed extending beyond the borders of the MIN. Axon collaterals were not seen in the MIN and only occasionally were perigeniculate collaterals observed. Other Y-cells in our sample possessed type B fusiform somata and bipolar dendrites. Two neurons in our sample do not readily fit into any of our three morphological classes of MIN cells.

Supported by USPHS Grant EY03038.

303.4 CELLULAR AND OPTIC TRACT AXONAL MORPHOLOGIES IN THE LATERAL GENICULATE BODY OF GALAGO SENEGALENSIS. M. Conley, G.R. Penny and I.T. Diamond. Neurobiology Program and Department of Psychology, Duke University, Durham, NC 27706.

This is a report of two studies aimed at further understanding the functional significance of the differences between lateral geniculate (GL) layers in Galago. The Galago GL is composed of three pairs of layers: layers 1 and 2 are magnocellular, layers 3 and 6 parvocellular and layers 4 and 5 koniocellular. In the first study we examined the morphology of GL relay neurons filled by HRP injections in the optic radiations. The results support the idea that each ipsilateral layer is matched with a contralateral layer. There are two principal cell groups in GL layers 1 and 2: one is large (300-500 μm^2), multipolar and has numerous radially oriented dendrites; the second is smaller (200-300 μm^2), pyramidal in shape and has numerous grape-like appendages at dendritic branch points. In layers 3 and 6 relay cells are medium-sized (150-300 μm^2) and fusiform in shape with polarized dendrites that arborize in a narrow band perpendicular to the long axis of the layer. Layers 4 and 5 contain neurons with small somata (100-250 μm^2) and numerous radial dendritic processes.

We also used the Golgi method to examine GL cells. These experiments confirmed the HRP results and also revealed a class of very small neurons (70-150 μm^2) in all GL layers that were not labeled in the HRP back-filled material. These cells had 2-3 stout primary dendrites from which fine processes branched into brush-like endings. We suggest that this cell class corresponds to the local circuit, GABAergic neurons described previously (Fitzpatrick et al., Neurosci. Abstr. 8:261).

In a second study we traced the pattern of termination of retino-geniculate fibers after HRP injections in the optic tract (TO). In general, TO fibers were divisible into large, medium and small groups, each ending in a corresponding large, medium and small cell GL layer. Layer 1-2 fibers were largest (2.5-4.5 μm), layer 3-6 fibers were intermediate (1.5-2.5 μm) and layer 4-5 fibers were thinnest (0.5-1.25 μm). The size and shape of an axon's terminal arborization was closely related to the morphology of the cells in its target layer. For example, layer 3-6 fibers terminate in tall, narrow columns whose width corresponds to that of the dendritic arborization of a layer 3-6 neuron.

Taken together the results suggest, first that the three pairs of GL layers in Galago represent largely independent visual pathways; but the results also reveal that the organization may be more complex than just three parallel pathways, since morphological subclasses of relay neurons can be found within a single pair of layers (e.g., in layers 1 and 2).

(Supported by NSF research grant BNS-8209081 and NIMH research grant 04849 (ITD) and NIMH predoctoral fellowship 08312 (GRP)).

303.5 FUNCTIONAL CLASSES OF NEURONS IN THE MONKEY'S LATERAL GENICULATE NUCLEUS HAVE DISTINCTIVE MORPHOLOGY . Charles R. Michael, Dept. of Physiology, Yale Medical School, 333 Cedar Street, New Haven, CT. 06510.

Intracellular recordings combined with horseradish peroxidase injections have revealed that the various physiological types of geniculate cells, as well as their retinal afferents, have specific anatomical characteristics. Most parvocellular neurons (Type 1) had center/surround receptive fields with small centers, gave sustained red/green opponent color responses and were found in layers 6 through 3. Generally, their dendrites were oriented perpendicular to the laminar borders, i.e. along projection lines. In many cases they reached across the width of a layer and sometimes into an interlaminar zone but never into an adjacent lamina. A few Type I cells were blue on center and were confined to layers 4 and 3. Their dendrites were also normal to the laminar edges but had wider lateral spreads, possibly related to their larger field centers.

Type II cells were blue on, yellow off and were also limited to layers 4 and 3. Their dendrites were parallel to the laminar borders, had extensive lateral distributions but never occupied the entire width of a layer. Their receptive fields were the largest of the parvocellular neurons. Type III cells, which exhibited no opponent color properties on white backgrounds, had radially symmetrical dendritic arbors (layers 6-3).

Contrast-sensitive magnocellular neurons gave transient discharges and had relatively large field centers. Their dendritic fields were radially symmetrical and very extensive, usually spanning the entire width of a layer. They always reached into the interlaminar zones and usually into the adjacent lamina. A single Type IV cell had a broad band on center with a red suppressive surround and a bipolar morphology with dendrites oriented perpendicular to the layers.

The terminal patterns of the different optic tract fibers were similarly distinctive. Arborizations of Type I fibers occupied a roughly cylindrical envelope which was normal to the laminar boundaries. Type II axons terminated across only part of a layer, arborizing primarily in a lateral direction. Fibers which terminated in a wide, circular fashion had Type III physiological properties. Axons destined for the magnocellular layers had very large terminal fields, usually extending across the full width of a lamina. (Supported by National Eye Institute Grant EY 00568).

303.6 ULTRASTRUCTURAL STUDY OF P-CELL/I-CELL RATIOS IN MONKEY DORSAL LATERAL GENICULATE NUCLEUS. P. Pasik, T. Pasik and J. Hámori*. Dept. Neurol., Mount Sinai Sch. Med., New York, N.Y. 10029, and Dept. Anat., Semmelweis Univ. Med. Sch., 1450 Budapest, Hungary.

The predominance of projective neurons (P-cells) of the Y type in the magnocellular (Mg) division, and of the X type in the parvocellular (Pv) division of the monkey LGN, makes this nucleus a prime target to investigate the synaptic relationships which may be responsible for the parallel processing of visual information. The functional properties of the two subsystems may result not only from the different classes of P-cells, but from variations in neuronal circuits with participation of presynaptic dendrites belonging to interneurons (I-cells).

In the present study, we quantified the proportion of I-cells and P-cells in each laminar type, by means of ultrastructural criteria based on the observation of acute retrograde changes in the LGN of monkeys (M. mulatta), 4 and 6 days after extensive cortical removals which included the entirety of areas 17, 18 and 19. Marked chromatolysis and diminution of rough endoplasmic reticulum were noted in 89 neurons of medium or large size, with rich cytoplasmic matrix, numerous large mitochondria, up to 0.7 μm in diameter and 4 μm long, and exhibiting only postsynaptic sites on the somata and dendrites. The changes consisted of separation of the polyribosomes into individual evenly dispersed granules, detachment of ribosomes from the rough endoplasmic reticulum, and increased number of lysosomes. These alterations were present in perikarya and in dendritic profiles recognized as belonging to these neurons on the bases of mitochondrial morphology, and the presence of junctions with dense filamentous material close to synapses made with retinal boutons. The abnormal occurrence of ribosomes in dendritic spines and axon initial segments were also noted. Seventeen other neurons showed none of such changes, and were characterized by a pale matrix, small dense mitochondria, up to 0.25 x 1.0 μm, and both postsynaptic and presynaptic sites on their somata and dendrites. It was concluded that the former and latter groups represented P-cells and I-cells respectively.

Using the preceding morphologic criteria, 250 neuronal somata containing nuclear profiles were identified in 391 and 293 random sections of Mg and Pv laminae respectively. I-cells comprised 15.6% of the neuronal population in the Mg division, and only 4.4% in the Pv layers.

The findings provide unequivocal evidence for the greater proportion of I-cells in the Mg division of the LGN. The ultrastructural criteria circumvent most objections raised to previous quantitative findings based on light microscopic examination of HRP labeled cells. The greater number of I-cells within neuronal circuits made by P-cells of the Y type may signalize the importance of the assumed inhibitory function of the interneurons in maintaining the transient nature of the Y-cell responses. This influence would be effected through the formation of triadic, serial and reciprocal synapses made by the I-cell presynaptic dendrites and perikarya.

Aided by NIH Grants #EY-01867 and NS-11631.

303.7 SEROTONIN IMMUNOREACTIVITY IN THE MONKEY DORSAL LATERAL GENICULATE NUCLEUS. Tauba Pasik, Pedro Pasik and Gay R. Holstein*. Dept. Neurol., Mount Sinai Sch. Med., CUNY, New York, N.Y. 10029.

Studies in lower mammals have implied that some interneurons of the dorsal lateral geniculate nucleus (LGNd) are serotoninergic. In addition, afferent fibers from the raphe nuclei have been described, and their serotoninergic nature suggested by histofluorescence and autoradiography. The availability of serotonin antibodies offered a more direct proof of the existence of these elements and indeed immunofluorescence demonstrated a medium to high density of such fibers in the LGNd of the rat (Steinbusch, 1981). We have used the unlabeled antibody PAP technique to identify serotoninergic elements in the LGNd of monkeys (M. fascicularis) at both the light and electron microscopic levels. Intracerebral injection of colchicine, dorsal to the LGNd,was used in some cases to maximize the labeling of neuronal somata. Vibratome sections of material perfused with buffered 4% pure formaldehyde and 0.25% glutaraldehyde were immunostained and osmicated before flat embedding in Epon-Araldite between plastic coverslips, thus allowing study of the same tissue at both levels. Light microscopy failed to reveal any immunoreactive somata. A low to medium dense plexus of fine, beaded fibers was apparent, with a gradient of density decreasing from magnocellular to parvocellular laminae. The fibers were up to 1 m in diameter with irregularly spaced varicosities. The arborization pattern was difficult to follow due to the plane and relative thinness of the sections. Similar features were noted in the ultrastructure. The label consisted of dense round particles, 40-60 nm in diameter, as well as granular material attached to outer mitochondrial membranes and microtubules. Profiles ranged from 0.5 to 1.5 m in cross sectional diameter, and occasionally showed thin myelin sheaths. Serial sections of obliquely cut fibers permitted following their course through the neuropil, and their varicose nature became apparent. They occupied a peripheral position in quasi-glomerular complexes, and in a few examples formed synapses with profiles identified as principal cell dendrites or presynaptic interneuron dendrites. The membrane specializations were asymmetrical with few instances of subjunctional dense bodies. In some of the labeled profiles, vesicles appeared clear and with a heavy dense outer coating.

The findings provide evidence for the positive identification of serotoninergic axonal elements in monkey LGNd, and do not support the existence of serotoninergic interneurons in this structure. The synaptic morphology suggests an excitatory effect on the postsynaptic partner, and the paucity of well defined junctions raises the possibility of an additional non-synaptic, probably modulatory, influence of these afferents on geniculate cells. Aided by NIH Grants # EY-01867 and NS-11631.

303.8 MORPHOLOGY OF THALAMOCORTICAL AXONS IN THE TURTLE, PSEUDEMYS SCRIPTA. S. B. Heller* (SPON: P. S. Ulinski). Dept. of Anatomy, Univ. of Chicago, Chicago, IL 60637.

Orthograde tracing techniques have indicated that the dorsal lateral geniculate complex in Pseudemys is reciprocally connected with a dorsal telencephalic region called dorsal cortex (Hall and Ebner, 1977) where thalamocortical synapses are confined to the outermost regions of the molecular layer (Colonnier and Ebner, 1975; Smith, et al., 1980). The present light microscopic study examines the morphology of individual axons within the geniculocortical path following HRP injections in the lateral thalamus in both in vivo and in vitro preparations.

Anterogradely filled axons were traced from the geniculate complex to the cortex in 100 um serial sections. Axons are smooth and are 2 to 0.5 um thick as they exit the geniculate complex. They form discrete fascicles, interdigitating with other thalamic efferents leaving the diencephalon principally via the dorsal peduncle of the lateral forebrain bundle. They decrease in diameter while travelling rostrolaterally without branching and bear varicosities in the striatum that are apposed to retrogradely filled neurons. Many axons leave the main fascicle of the geniculocortical path at the level of the pallial thickening, where they acquire varicosities and turn obliquely toward the ventricular surface. Varicosities, about 2 x 2 um in size, are numerous in the pallial thickening where they are apposed to the somata and proximal dendrites of retrogradely labeled pyramidal neurons. The major fascicle of axons continues dorsolaterally into the molecular layer of dorsal cortex where they continue as very thin fibers bearing small (1 x 1 um) varicosities. The major terminal zone ends precisely at the border of dorsal cortex with pallial thickening. A small proportion of thicker axons travel through the entire extent of the dorsolateral telencephalon with few varicosities.

These observations indicate that the geniculocortical path is substantially more complex than indicated by earlier studies. Thalamocortical axons bear en passant varicosites in the striatum and terminate in two distinct regions of the pallium. They appear to synapse upon the somata and proximal dendrities of corticothalamic neurons in the pallial thickening and upon the distal dendrites of neurons in dorsal cortex, which tend not to be retrogradely labeled in thalamic HRP injections. (Supported by PHS Grant NS 12518)

303.9 MICROCIRCUITRY IN THE SUPERFICIAL GRAY LAYER OF THE CAT SUPERIOR COLLICULUS, R. Ranney Mize, Department of Anatomy, University of Tennessee Center for the Health Sciences, Memphis, TN 38163.

The superficial gray layer of the cat superior colliculus receives afferents from the retina and visual cortex and contains a variety of cell types, some of which project to the lateral posterior nucleus and the ventral and dorsal lateral geniculate nuclei. Our laboratory has been identifying specific cell types and tracing their connections using neurotransmitter labels, retrograde tracing with horseradish peroxidase, and serial section reconstruction. Using these approaches, we have begun to map the synaptic microcircuitry of this region of the midbrain. Seven cell types have been identified within the superficial gray layer (Mize, et al., 1982). Four of these are long projection neurons (Harrell, et al., 1982). Stellate cells have a high synaptic density. Many project to the lateral posterior nucleus (LP), a few to the dorsal and ventral lateral geniculate nuclei (LGN). These cells receive input on their proximal dendrites from visual cortex but not the retina. Vertical fusiform cells also have a high synaptic density. They receive input from both visual cortex and retina and project in about equal numbers to LP and LGN. Type II granule cells have a moderate synaptic density, get input from visual cortex, and project largely to LGN. The axons of these cells get synaptic input from other axon terminals. Horizontal II cells project only to LP. We do not yet know their synaptic input.

Three types of interneuron have also been identified in the superficial gray layer. The Horizontal I cell has a low synaptic density, a high percentage of cortical input, and accumulates GABA and muscimol intensely. It has presynaptic dendrites which form dendrodendritic synapses, but apparently lacks an axon. The Granule I cell has a moderate synaptic density, limited cortical input, and also accumulates GABA and muscimol. It has an axon which we believe gives rise to GABA-accumulating flattened vesicle axon terminals which ramify locally within the superficial gray. These terminals contact 4 of the cell types within this layer of the colliculus. The Type III granule cell has dendritic spines containing synaptic vesicles and likely participates as the intermediate element in serial synapses involving cortical terminals and the dendrites of projection cells.

The geometries and connections of these cell types suggest a rudimentary circuitry which has important functional implications. The Horizontal I cell, for instance, is probably involved in spatial inhibition which must extend across much of the collicular surface. The Granule I cell likely participates in temporal inhibition which is spatially localized. Other structural relationships also have functional significance. (Supported by NIH Grant EY-02973).

303.10 ABNORMAL RETINAL GANGLION CELL PROJECTIONS TO PRETECTAL NUCLEI AND THE ACCESSORY OPTIC SYSTEM IN MICE WITH HYPOPIGMENTED RETINAL PIGMENT EPITHELIUM AND ABNORMAL OPTOKINETIC NYSTAGMUS. Marion W. Pak* and Lawrence H. Pinto. Department of Biological Sciences, Purdue University, West Lafayette, IN 47907.

Retinal ganglion cell projections to nuclei of the pretectum and accessory optic system of mice with normally pigmented retinal pigment epithelium (RPE) and normal optokinetic nystagmus were compared with the projections of hypopigmented RPE mice having few, if any, normal optokinetic nystagmus movements.

Mice of 12 hypopigmented genotypes and 5 strain-matched pigmented genotypes were injected intraocularly with 20 μCi of ^{3}H-proline and allowed to survive for up to 12 days. They were then perfused intracardially, and the brains and eyes were processed for autoradiography.

The structures examined in detail were the nucleus of the optic tract (NOT), the olivary pretectal nucleus (OP), the posterior pretectal nucleus (PP), the dorsal terminal nucleus (DTN), the lateral terminal nucleus (LTN) and the medial terminal nucleus (MTN). Principal fasciculi leading to these nuclei were also examined. In many hypopigmented RPE animals the label seen in the contralateral dorsal pretectal PP, OP, NOT, DTN area appeared to be decreased. The configuration of the label also appeared to be somewhat altered. In particular, both the cross-section and length of the NOT label tended to be reduced in hypopigmented animals.

By examining 10μ serial sections through the area of the NOT, we saw what appeared to be tiny but consistent ipsilateral NOT label in both pigmented and hypopigmented mice where the label was heavy and the autoradiogram background was low.

One of the principal fasciculi going from the retina to the medial terminal nucleus is the inferior fasciculus of the accessory optic tract (AOT). Its pathway goes from the tip of the medial point of late-crossing axons in the optic chiasm, through the ventral aspect of the brain, and eventually to the ventromedial and dorsomedial aspects of the medial terminal nucleus. In certain hypopigmented mice this contralateral pathway seems attenuated.

Finally, although some contralateral projections to the nuclei of the AOT appeared attenuated, in some cases the ipsilateral label to these AOT nuclei appeared to be increased.

The apparent anomalies of these pathways are of interest to us because previous researchers have shown the NOT, DTN, and MTN to contain directionally selective cells that are associated with optokinetic nystagmus movements.

303.11 RESPONSES TO VISUAL STIMULATION IN SINGLE CELLS IN THE NUCLEUS OF THE OPTIC TRACT (NOT) DURING OPTOKINETIC NYSTAGMUS (OKN) IN THE AWAKE CAT. K.P. Hoffmann and H.P. Huber*, Abt. Vergl. Neurobiologie, Universität Ulm, Postfach 4066, D-7900 Ulm, GFR.

A lot of indirect evidence suggests a role of single neurons in the NOT of mammals in mediating OKN. Most of the evidence derives from the analysis of single cell responses to visual stimulation in the anaesthetized preparation and on the analysis of OKN in normal animals or in animals with pretectal or cortical lesions. We took the direct approach in recording from NOT neurons in awake cats with search coils implanted around their eyes to measure eye movements in a magnetic field during optokinetic stimulation. As in the NOT of anaesthetized cats the cells preferred large area stimuli, responded well to very low velocities (< 1°/s) were direction specific for horizontal movement and were mostly binocularly activated. So far we have analyzed the relationship to OKN in 20 cells from 2 animals. All cells responded to retinal slip over a range from less than 1°/s to more than 100°/s (best response at about 10 - 20°/s). The discharge rate (spontaneous: 5 - 70 spikes/s) was not modulated during optokinetic afternystagmus or during the vestibulo ocular reflex in the cark. Electrical stimulation through the recording electrode (pulse width 1 ms, frequency 60 Hz, maximal amplitude 0.5 mA) elicited eye movements in a clear OKN pattern as if the preferred stimulus was presented to the cells.

In summary NOT cells provide a direction specific signal about retinal slip during OKN. In a normal cat OKN gain was always smaller than 1.0 and cells in the left (right) NOT would discharge at a rate above spontaneous activity as long as the stimulus moved leftward (rightward). Electrical stimulation at the recording site in the left (right) NOT elicited OKN with leftward (rightward) directed slow phases.

303.12 AUDITORY PHYSIOLOGICAL PROPERTIES OF THE NEURONS IN THE SUPERIOR COLLICULUS OF ECHOLOCATING BATS P.H.-S. Jen, X.D. Sun,* and S.Q. Zhang,* Division of Biological Sciences, The University of Missouri, Columbia, Missouri 65211.

Although the visual centers of insectivorous bats are generally poorly developed, the superior colliculus (SC) is an exception. To study the functional role of the SC during a bat's echolocation, responses of 167 SC neurons of the big brown bat, Eptesicus fuscus to pure tone pulses and frequency-modulated (FM) stimuli were studied. The neurons fired only a few impulses to acoustic stimuli. Their latencies were between 4 and 20 msec with most of them below 12.5 msec. Threshold curves were either narrow, intermediate or broad according to the widest frequency band which each neuron tuned. Q_{10-dB} values of these threshold curves ranged between 1.21 (BF=53.5 kHz) and 67.9 (BF=69.9 kHz), but the majority were below 20. Minimum thresholds (MT) of these neurons to pure tone pulses and one octave upward and downward sweeping FM stimuli were compared. These neurons generally had their lowest MTs to downward sweeping FM stimuli and their highest MTs to pure tone pulses. An upward sweeping FM stimulus was generally less effective than its corresponding downward sweeping FM stimulus in eliciting a response from a neuron. A series of intensity-rate function of 16 neurons was measured with both pure tone pulses and upward and downward sweeping stimuli. There was no correlation between the type of intensity-rate function obtained and the type of acoustic stimulus used. The SC auditory neurons were not tonotopically organized. However, neurons isolated from the same layer appeared to have comparable best frequencies. (Work supported by NSF BNS 80-07348 and NIH USPH-1-K04-NS-0043 to P.H.-S. Jen.)

304.1 COMPARISON OF THE AFTEREFFECTS OF ACTIVITY BETWEEN NODES OF RANVIER. L. R. Carley* and S. A. Raymond (SPON: L. S. Frishkopf). Research Laboratory of Electronics, MIT, Cambridge, MA 02139.

Nodes of Ranvier from a single axon, from whole nerve, and from different nerves have been compared using the aftereffects of impulse activity on the threshold as a metric. Threshold was measured using the method of threshold hunting as described by Raymond (J. Physiol. 290:273-303, 1979). Excised sciatic nerves from Rana pipiens were bathed in Boyle Conway Ringer's solution using a bicarbonate buffer (at pH 7.2). Focal stimulation was applied via one of 10 unipolar Ag/AgCl electrodes of .4mm diameter located each 2mm along the upper third of the nerve trunk. The distal end of the nerve fiber was teased apart and single axons were recorded using a suction electrode. Different nodes from the same axon were selected by stimulating from different electrodes. Measures for the superexcitable phase of the aftereffects characterizing the peak value, the area of the superexcitable phase, and the decay time constant were developed. Similarly, measures for the depression phase of the aftereffects characterizing the onset time, the peak value, and the decay time were developed. For nodes selected from across the entire nerve bundle there is a wide range in the measures of superexcitability (on the order of 1:10) and an extremely wide range in the measures of depressibility (on the order of 1:1000). In experiments to date the range of the measures for both superexcitability and depressibility between nodes along the same axon is much smaller.

It seems likely that the similarity from node to node along a fiber implies that the cell regulates mechanisms influencing excitability. The large differences in activity dependence between nodes from different fibers having the same conduction velocity in the same nerve trunk may reflect specializations of recovery mechanisms to handle activity patterns in fibers coding and transmitting different types of information.

This work was supported by USPHS grant 30160 and a Whitaker Health Sciences Fund Fellowship to LRC.

304.2 IS AXONAL CONDUCTION BLOCK RESPONSIBLE FOR FREQUENCY-FOLLOWING FAILURE IN THE CAT CUNEATE NUCLEUS? R. J. Weinberg and A. L. Towe. Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195.

Iterative electrical stimulation of a peripheral nerve at progressively increasing rates usually results in failure of synaptic transmission before decline in amplitude of the nerve compound action potential. One possible explanation is transmission failure at low-security regions of the terminal axonal arbor, such as branch points. We tested this possibility by recording single units with glass microelectrodes in the second cervical segment of the dorsal funiculus and in the region of the cuneate nucleus during iterative electrical stimulation of peripheral nerves.

We defined Fmax, the maximum frequency following, as that peripheral nerve stimulus frequency above which more than 10% of stimuli failed to evoke a spike in the recorded unit. Median Fmax at stimulus intensity 3 times threshold (3xTh) for dorsal funicular axons was five times higher than at 1.2 x Th, implying failure at the site of stimulation for near-threshold stimulus strengths. Presynaptic axons isolated in the region of the cuneate nucleus showed Fmax at 1.2 x Th very similar to that of the dorsal funicular sample. However, median Fmax at 3xTh was only three times higher for these axons, raising the possibility of axonal conduction failure in the first cervical segment. Fmax for presynaptic axons at 3xTh did not differ as a function of rostro-caudal recording position within the cuneate nuclear region, nor was there a detectable trend for presynaptic axons recorded close to cells to show a lower Fmax than those recorded farther away. Thus, there was no evidence for branch point failure within the nucleus. On the other hand, the median Fmax for these presynaptic axons was more than 2.5 times higher than that for cuneo-thalamic projection cells, at both weak and strong stimulus intensities.

Because most of these cells are monosynaptically driven by dorsal funicular axons, the above observations suggest that axonal branch point failure plays little role in defining Fmax for cells in the cuneate nucleus. This and other evidence suggests that the crucial mechanisms of failure are at or near the synapse itself. If axonal conduction block is indeed irrelevant to synaptic failure in the cuneate nucleus--a system known for high-security synaptic transmission--then doubt is cast on hypotheses proposing a role for axonal block in other, more integrative systems.

This work was partially supported by NIH grant #NS05136.

304.3 A NEW APPROACH TO STUDY SYNAPTIC TRANSMISSION IN THE ISOLATED FROG SPINAL CORD. S. Glusman, Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

The analysis of synaptic physiology in the vertebrate central nervous system (CNS) is a technically demanding problem. Because of the anatomical complexity of the CNS questions relating to individual synaptic contacts, the separation of pre and postsynaptic events and their modulation by use, transmitters, or neurohormones are difficult to analyze. In this communication we propose a method to study synaptic transmission in a restricted number of nerve terminals in the isolated frog spinal cord and to perform quantal analysis of transmitter release on these terminals. A double barrelled micropipette (NaCl for recording, $CaCl_2$ for injection) is inserted into the cord and a dorsal root or a peripheral nerve is stimulated. The experimental plan is: 1) to localize a synaptic region of interest by extracellular field potential recording within the spinal cord; 2) to eliminate all synaptic activity by removing Ca^{++} from the bathing fluid; and 3) to restore synaptic activity in a selected group of nerve endings by microinjection of Ca^{++} through the Ca-filled barrel of the double barrelled micropipette. The extracellular field potential recorded in Ringer solution consist of: (1) an early diphasic (positive-negative) potential, corresponding to the activation of presynaptic nerve terminals; (2) a prolonged negative potential with two components, a synchronous negative component likely to be related to the monosynaptic activation of postsynaptic cells and a late and prolonged negativity probably due to the activation of polysynaptic pathways. Both components of this latter potential disappear when Ca^{++} in the medium is replaced by Mg^{++}. When Ca^{++} is iontophoretically injected (1-5 nA) the monosynaptic component of the intraspinal field potential (field-epsp) reappears. When the position of the micropipette and the injection of Ca^{++} are carefully adjusted, successive stimulation of sensory nerve terminals generate a field-epsp whose amplitude fluctuates from trial to trial and, on occasions, fails to evoke a postsynaptic response. An amplitude histogram of a series of field-epsps shows regularly occurring peaks, each peak occurring as a multiple of a unitary amplitude. A good correlation was obtained between the observed amplitude distribution of the field-epsps and that predicted by the Poisson theorem. We have used this method to examine the suggestion that GABA serves in the spinal cord as a neurotransmitter involved in presynaptic inhibition. The application of GABA ($5x10^{-4}$M) reduced the field-epsp in certain sets of afferent fibers. Preliminary quantal analysis suggests that GABA causes a reduction in the mean number of quanta released by the presynaptic impulse as would be expected for a substance producing presynaptic inhibition. (Supported by NIH).

304.4 RECONSTITUTION OF A FUNCTIONAL PRESYNAPTIC MEMBRANE : THE OPERATOR PROTEIN. M. ISRAËL*, B. LESBATS*, N. MOREL*, R. MANARANCHE*. (SPON : C. SOTELO) Department of Neurochemistry, Lab. of Cellular Neurobiology, C.N.R.S., 91190 Gif-sur-Yvette (France).

Soon after the finding that synaptic vesicles we had isolated from Torpedo electric organ contained most of the total acetylcholine (ACh), we had to admit that the non vesicular, free ACh (30% of total) was the physiologically active pool. It was depleted by stimulation and renewed by the cytosolic enzyme choline-acetylase. Free ACh variations were directly correlated to the ACh output. Later we have developed a procedure for preparing synaptosomes from that tissue. With the aid of our new chemiluminescent ACh assay we gained many new informations. (The procedure is based on the hydrolysis of ACh and on the oxidation of choline by choline oxidase, the H_2O_2 generated gives light with luminol + peroxidase). ACh release was continuously monitored from stimulated synaptosomes and the decrease of free ACh directly evaluated after disrupting the synaptosomes. Again vesicular ACh was stable. The next step was to show that sacs of presynaptic membrane filled with ACh released it in proportion to the calcium influx and to the internal ACh concentration. We are now able to reconstitute from a lyophilised presynaptic membrane powder a fully functional proteoliposome. The reconstituted membrane is equipped with the same intramembrane particles found in the synaptosomal membrane. The only constant ultra structural change found in the synaptosomal and reconstituted membranes when they release ACh is a rearrangement of intramembrane particles. Presently we have separated from a cholate extract of synaptosomal membranes the substance (named operator) which gives to the liposomes their ability to release ACh. This presynaptic membrane protein has a molecular weight between 600,000 and 200,000 d. After SDS electrophoresis, subunits at 90,000, 60,000 and 40,000 are found in the active fraction. The operator protein, now characterised, permits the translocation of ACh upon calcium action.

304.5 MANGANESE FLUXES AND MANGANESE-DEPENDENT NEUROTRANSMITTER RELEASE IN PRESYNAPTIC NERVE ENDINGS ISOLATED FROM RAT BRAIN. D.A. Nachshen and P. Drapeau*. Dept. Physiology, Univ. Md., Sch. of Med., Baltimore, MD 21201.

The uptake and efflux of ^{54}Mn and ^{45}Ca, and the release of dopamine (DA) were measured in pinched-off presynaptic nerve terminals (synaptosomes) isolated from rat brain.

Mn and Ca uptake are both increased when either forebrain or striatal synaptosomes are depolarized in K-rich solutions. The time-courses of K-stimulated Mn and Ca uptake are similar: There is initially a high rate of divalent cation accumulation lasting 1-3 sec, that gradually decreases. Several Ca-channel blockers (Mg, Sr, Ba, Co, Ni, and La) suppress the K-stimulated uptake of Mn and Ca to a similar extent. Mn uptake saturates, and Ca uptake is blocked, as the external Mn concentration is increased.

There is a decreased efflux of Ca, but not of Mn, from synaptosomes when the external Na concentration is reduced. The Na-dependent efflux of Ca is diminished by external Mn, but it is unaffected when the synaptosomes are Mn-loaded.

The rate of tritium-labeled DA release from striatal synaptosomes in low K, non-depolarizing, solutions is less than 0.001 sec^{-1}, in the absence or presence of Mn and Ca. The addition of 1 mM Mn to depolarizing solutions increases the rate of DA release by 40%; the rate of release remains at this level for at least 30 sec. The addition of 1 mM Ca to depolarizing solutions increases the rate of DA release nearly 100-fold during the first sec; thereafter, the rate of release declines rapidly. The addition of Mn or Ca to depolarizing solutions is also able to increase the release of 5-hydroxytryptamine and of γ-aminobutyric acid. Ni and Mg reduce K-stimulated DA release that is dependent on either Mn or Ca. This reduction is probably due to a reduction in the K-stimulated uptake of Mn and Ca.

These results indicate that Mn competes with Ca for passage through the volage-dependent Ca channels in brain presynaptic nerve terminals. Mn can support neurotransmitter release from the nerve terminals, but far less effectively than Ca.

D.A.N. was supported by NIH grant NS 16461. P.D. was supported by a Fellowship from the Medical Research Council of Canada, and by NIH grant NS 16101 to Prof. M.P. Blaustein.

304.6 β-LEPTINOTARSIN-H: AN ACTIVATOR OF PRESYNAPTIC CALCIUM CHANNELS. R.D. Crosland, T.H. Hsiao*, and W.O. McClure. Section of Neurobiology, University of Southern California, Los Angeles, CA 90089 and Department of Biology, Utah State University, Logan, UT 84322.

A neuroactive protein, β-leptinotarsin-h, has been purified to near homogeneity from the hemolymph of the beetle Leptinotarsa haldemani by column chromatography. β-Leptinotarsin-h has a molecular weight of 57,000. Rat brain synaptosomes incubated with appropriate radioactive precursors release acetylcholine (ACh), norepinephrine, and 4-aminobutyrate when exposed to β-leptinotarsin-h, but do not release lactate dehydrogenase. Release of ACh has been examined in some detail. Release of ACh varies with the concentration of β-leptinotarsin-h in a rectangular hyperbolic fashion. One-half maximal release is stimulated by a concentration of 50 ng/ml. Altering the ionic composition of the bathing solution affects release in a manner which suggests that neither Na^+ nor K^+ channels are affected by β-leptinotarsin-h, but that β-leptinotarsin-h acts to increase permeability to Ca^{2+}. Varying the concentration of Ba^{2+}, Sr^{2+}, Co^{2+}, and Cd^{2+} indicates that β-leptinotarsin-h acts to open the voltage-sensitive presynaptic Ca^{2+} channel. β-Leptinotarsin-h may be a useful tool for studying the Ca^{2+} channel associated with the release of neurotransmitters.

304.7 PRESYNAPTIC NEUROTOXIN RECEPTORS: A STUDY USING ß-BUNGAROTOXIN. H. Rehm* and H. Betz (SPON: European Neuroscience Association). Dept.Neurochemistry, Max-Planck-Institute for Psychiatry, Martinsried, FRG.

ß-Bungarotoxin (ß-BTX) is a presynaptically active neurotoxin from the venom of B. multicinctus which inhibits neurotransmitter release at the motor nerve terminal. It was previously shown that this neurotoxic phospholipase A_2 (M_r=21,000) displays a selective cytotoxicity upon central neurons. In chick retina, ß-BTX preferentially destroys GABAergic and cholinergic nerve cells (Rehm, H. and Betz, H., Brain Research, 250: 309, 1982). In order to characterize the membrane molecule mediating the action of ß-BTX the toxin was labeled with ^{125}I, and its specific binding to brain membranes was determined (Rehm, H. and Betz, H., J.Biol.Chem., 257: 10015, 1982). The ß-BTX binding site was found to be a minor protein component of the nerve cell membrane exhibiting high affinity towards the toxin. Because of its potentially important role in neurosecretion, the ß-BTX binding protein was further analyzed through photoaffinity crosslinking of ^{125}I-labeled ß-BTX to brain membranes and subsequent analysis of the membranes by SDS-gel electrophoresis and autoradiography. A labeled band of M_r = 116,00 was detected by these techniques. The labeling of this band was inhibited under conditions previously shown to block the specific and saturable binding of ^{125}I-ß-BTX to the membrane fractions. It therefore is concluded that the ß-BTX binding site of the neuronal membrane contains a polypeptide of MW 95,000. Recently, conditions for the solubilization of the ß-BTX binding site have been established. Supported by the Deutsche Forschungsgemeinschaft.

304.8 FACTORS INFLUENCING THE TIME COURSE OF DECAY OF END-PLATE CURRENTS IN PRESENCE OF PROSTIGMINE. M.I. Glavinovic. Departments of Anaesthesia Research & Physiology, McGill University, Montréal, Québec H3G 1Y6, Canada.

It has been observed before that the time constants of decay of both miniature end-plate currents (MEPCs) and end-plate currents (EPCs) become prolonged in the presence of prostigmine. This has been explained on the grounds that due to block of cholinesterase activity molecules of ACh bind repetitively and in case of EPCs with large quantal contents there is also spatial overlap of quantal events. Furthermore in the presence of prostigmine if two stimuli are applied in quick succession (2-10 msec) the second EPC decays more slowly than the first presumably not only due to facilitated release (which results in greater spatial overlap of quantal events) but also due to ACh that remains from the previous release-lingering ACh. These experiments are an attempt to separate these contributions.

MEPCs and EPCs were studied in an unparalyzed frog "cut" cutaneous pectoris prostigmine treated (10^{-6} g/ml) preparation at room temperature (19-20° C) using voltage clamp technique. The nerve was stimulated with low frequency of stimulation (0.2-1 Hz), twin pulses at varying intervals (5-30 msec) and short tetanic trains (10-20 pulses, 50-100 Hz). When the nerve is stimulated at low frequency and EPCs are varied over a wide range of amplitudes by varying Ca/Mg ratio in the medium it is observed that EPCs are prolonged more than average MEPCs. This difference increases with increase in the amplitude of EPCs, for low amplitudes little but then progressively more. The τEPC vs EPC relationship tells us how spatial overlap of quantal events contributes to prolongation of EPCs. It also tells us how much EPCs should be prolonged if they are prolonged only because of increased repetitive binding and overlap of synaptic events. Using this relationship it can be shown that the second of two EPCs applied a brief interval after the first is prolonged not only due to increased spatial overlap of quantal events (caused by increase in quantal content-facilitation) but also due to lingering ACh as well. Moreover during short tetanic train when EPC amplitudes first increase and then decrease, but τEPCs continue to increase, it is shown that increase in τEPCs is predominately due to lingering ACh.

(Supported by MRC and MDA of Canada).

304.9 PRESYNAPTIC Ca^{++} ACCUMULATION IS THE MECHANISM OF POST-TETANIC POTENTIATION IN APLYSIA: EVIDENCE FROM THE Ca^{++}-INDICATOR DYE ARSENAZO III. E. Shapiro, J. Connor, R. Kretz.* Center for Neurobiol. & Behav., College of Physicians & Surgeons of Columbia Univ., N. Y., N. Y., and Bell Laboratories, Murray Hill, N. J.

Post-tetanic potentiation (PTP) is a form of synaptic plasticity whereby high-frequency stimulation of a presynaptic cell leads to increased transmitter release. PTP occurs at the L10-follower cell synapses in the abdominal ganglion of Aplysia californica. Previous voltage-clamp studies on this synapse (Kretz et al., PNAS, 79, 5430, 1982) correlated the time-course of a slow outward Ca^{++}-dependent K^+ current with PTP, supporting the residual Ca^{++} hypothesis of PTP, which states that intracellular Ca^{++} concentration increases during tetanic stimulation and contributes to enhanced transmitter release following the tetanus during the course of its removal from the presynaptic terminals. To provide more direct evidence for this mechanism, we iontophoretically injected the Ca^{++}-sensitive indicator dye Arsenazo III into presynaptic cell L10 and monitored changes in intracellular Ca^{++} concentration during PTP. Injected cells were voltage-clamped in seawater containing 30 μM TTX to block Na^+ currents at a holding potential of -40 or -50 mV. Fifty msec depolarizing pulses to +10 or +20 mV delivered tetanically (100 pulses at 4 Hz) gave rise to an Arsenazo signal which increased during the tetanus and declined after the tetanus with a time-course which could be described as the sum of two exponentials, a fast decay (τ_f = 6.2 ± 3.7 sec; n=9) and a slow decay (τ_s = 55.6 ± 18.9 sec; n=10). These kinetics are the same as those for PTP and the slow Ca^{++}-dependent K^+ current we previously reported (Kretz et al., 1982). Injection of Arsenazo blocked the fast component and reduced the slow outward component of the post-tetanic Ca^{++}-dependent K^+ current. The outward current that remained after Arsenazo injection decayed with a time course similar to the slow phase of the Arsenazo signal.

In five experiments we recorded from post-synaptic cells while monitoring Arsenazo absorbance presynaptically. In these experiments tetanic stimulation of L10 caused PTP of 150-300%. This PTP decayed with a single slow exponential time course similar to the slow time course of decay of the Arsenazo signal (τ = 80.0 ± 34.1 sec; n=5).

If L10 is tetanically stimulated in low Ca^{++} SW (0mM Ca^{++} 4mM EGTA) or in solutions containing Cd^{++} (4 mM), a Ca^{++}-channel blocking agent, no increase in the Arsenazo signal is seen even if Na^+ channels are not blocked with TTX (n=4), suggesting that tetanic stimulation increases intracellular Ca^{++} by allowing Ca^{++} to enter from the external medium through voltage-activated Ca^{++} channels.

These results further strengthen the residual Ca^{++} model of PTP. In addition, use of the Ca^{++} indicator dye Arsenazo III allows rough quantitative values to be calculated for the rise in intracellular Ca^{++} during tetanic stimulation. Our tetanus increased intracellular Ca^{++} concentration by 1.28 ± 0.60 x 10^{-6}M (n=7).

304.10 INTRACELLULAR STUDIES OF HIPPOCAMPAL FREQUENCY POTENTIATION: EVIDENCE FOR A PRESYNAPTIC, RESIDUAL CA2+ MECHANISM. T.A. Pitler* and P.W. Landfield (SPON: M. Levitt). Dept. of Physiol. & Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103.

Previous studies by Katz and Miledi (1968, J. Physiol.), and Rahamimoff (1968, ibid), among others, have indicated that residual Ca^{2+} accumulation in terminals underlies posttetanic and frequency potentiation at the neuromuscular junction. Recently, a correlation between potentiation and a presynaptic Ca^{2+}-activated K^+ conductance was found in Aplysia, supporting this view (Kretz, Shapiro, Kandel, 1982, PNAS). However, there is still no clear evidence that the robust frequency potentiation (FP) described in hippocampus (e.g., Andersen & Lomo, 1967, Prog. Brain Res.) depends on similar mechanisms. Since hyperpolarization of the postsynaptic cell occurs during repetitive stimulation (e.g., Schwartzkroin, 1975, Brain Res.; MacVicar & Dudek, 1979, ibid), increased postsynaptic driving force could account for the increase in EPSP amplitude. Alternatively, an increase in postsynaptic input resistance could account for FP of the EPSP.

To examine these possibilities, we performed a detailed quantitative analysis of EPSP amplitude, membrane conductance and membrane potential in CA1 pyramidal cells from hippocampal slices in 16 young-mature Fischer rats, before, and at multiple time points during, 4 min of 10 Hz stimulation of the Schaffer collaterals. In addition, we compared the effects of orthodromic and antidromic 10 Hz stimulation below and above the threshold for a population spike. Slices were bathed in either high (6.5 mM) or normal (4 mM) K^+ media. The effects of 10 Hz stimulation were also examined while recording from glial cells.

The data show that during 10 Hz stimulation of neurons, hyperpolarization occurs rapidly, is followed by depolarization at 15-30 sec of stimulation and then by renewed hyperpolarization over the 4 min. EPSP potentiation is not related to these changes, and in fact is maximal in the 15-30 sec period. However, a dramatic increase in input conductance also occurs during stimulation, the timecourse of which closely corresponds with that of EPSP potentiation. Moreover, both potentiation and conductance changes are greater in low K^+ media. Our data from glial cell and other experiments indicate that the membrane changes are the result of increased K^+ conductance, and several labs have shown that prolonged increases in K^+ conductance are Ca^{2+}-dependent in hippocampus.

The close correlation of the timecourse of FP with that of a likely Ca^{2+}-dependent process (K^+ conductance), and the fact that several postsynaptic factors can be ruled out as the basis for FP of the EPSP (e.g., hyperpolarization, increased resistance), strongly suggest that frequency potentiation of hippocampal EPSPs depends upon residual Ca^{2+} intracellularly, and moreover, that this is largely a presynaptic phenomenon. (Supported by AG 01737)

304.11 BLOT ANALYSIS WITH APLYSIA ^{125}I-CALMODULIN TO IDENTIFY NEURONAL PROTEINS REGULATED BY Ca^{++}. T. Saitoh and J.H. Schwartz. Center for Neurobiology & Behavior, N.Y. State Psychiatric Institute and the College of Physicians & Surgeons of Columbia University, New York, N.Y. 10032.

Ca^{++} has been shown to influence the activities of certain forms of vertebrate adenylate cyclase, phosphodiesterase, and a phosphoprotein phosphatase. In addition, some cAMP-dependent protein kinases bind calmodulin. In mollusks, cAMP-dependent protein phosphorylation has been implicated in a variety of neuronal activities that are mediated by changes in the functioning of ion channels, and this includes the presynaptic facilitation in identified sensory neurons that underlies sensitization of the gill withdrawal reflex in Aplysia. Because several of the molecules involved in the presynaptic facilitation might be regulated by Ca^{++} and bind calmodulin, we purified and radioiodinated Aplysia calmodulin and undertook the characterization of Ca^{++}/calmodulin binding proteins in Aplysia nervous tissue. Using blot analysis, we detected 14 calmodulin binding proteins in extracts of the abdominal ganglion. All 14 labeled components are present in each of the major central Aplysia ganglia, but their relative proportions differed characteristically from one ganglion to another. When neural components are extracted with nonionic detergents in 2M glycerol, we obtain a cytoplasmic and a membrane-cytoskeleton fraction. Fractionation studies indicate that two of the calmodulin binding proteins are cytoplasmic, 10 are loosely associated, and two are tightly bound to the membrane-cytoskeleton complex. We wished to see how these components might be altered when the synthesis of cAMP is stimulated. Treatment of isolated ganglia with serotonin, which has previously been shown to increase the content of cAMP within neurons, or with dibutyryl cAMP, causes the partial dissociation of only one of the loosely associated proteins which has a M_r of 55,000. In extracts in the presence of glycerol and cAMP, the component is also dissociated from the membrane-cytoskeleton complex, but only if ATP is also present. A cAMP-dependent phosphorylation of an undetermined protein appears to control the subcellular distribution of the 55,000 M_r calmodulin binding protein.

305.1 **LOSS OF ACETYLCHOLINESTERASE (AChE) IN THE BASAL FOREBRAIN OF ALZHEIMER DISEASE PATIENTS.** R. Jacobs* and L.L. Butcher. Department of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024, U.S.A.

Forty-five years ago, Hassler [1938, J. Psychol. Neurol. **48:** 387-476] observed loss of neurons in the nucleus of the substantia innominata (SI), sublenticular part, of an 84-year old human showing no obvious neurologic or psychiatric pathology. Since that time, but particularly recently, numerous reports have appeared suggesting a correlation between loss of cells in the basal forebrain with processes associated with normal aging and with the neuropathology of age-related disorders. Because many of the somata associated with the SI are cholinergic and because decrements in cholinergic indices are observed in various regions of the telencephalon in patients diagnosed as having Alzheimer's disease or senile dementia of the Alzheimer type (SDAT), the hypothesis has been advanced that cholinergic mechanisms involving the basal forebrain are importantly involved in cognitive functions and dysfunctions, particularly those associated with aging [review: Bartus et al., 1982, Science, **217:** 408-417]. Reanalyzing Nissl material from Alzheimer's disease patients, Whitehouse et al. [1982, Science, **215:** 1237-1239] reported loss of cell bodies in what they called the nucleus basalis of Meynert, but their findings could not be confirmed by Perry et al. [1982, Neurosci. Lett. **33:** 311-315]. Although results are preliminary, we provide in this abstract additional data concerning possible decrements in putative cholinergic neurons in the basal forebrain of Alzheimer's disease and SDAT patients.

Brain tissue from autopsied humans (4 cases of Alzheimer's disease-SDAT, 1 age-matched control, 1 young schizophrenic, 1 young normal control) was received from the National Neurological Brain Bank (Wadsworth VA Medical Center, Los Angeles, CA). Sections 60 μm thick were cut through the SI, and successive sections were stained for AChE alone, for Nissl substance alone, or for both AChE and Nissl on the same tissue section. Cell counts were made by two categories of raters, one blind to the diagnosis and the other cognizant.

Analyses of AChE-stained material revealed a marked reduction of enzyme activity in the SI of Alzheimer's disease-SDAT patients compared to the young normal control and the young schizophrenic. Although appreciable AChE activity was detected in the SI of the age-matched control, levels appeared lower than that seen in the younger subjects. Cell counts on Nissl material revealed an approximately 75% decrease in the number of somata in the SI of Alzhemier's disease-SDAT patients compared to remaining controls ($p < 0.005$). No significant difference was found among the 3 control subjects. The reasons for the discrepancy between this study and that of Perry et al. (vide supra) remain to be elucidated.

305.2 REGIONAL BRAIN CONCENTRATIONS OF NEUROTENSIN, THYROTROPIN-RELEASING HORMONE AND SOMATOSTATIN IN ALZHEIMER'S DISEASE C.B. Nemeroff, G. Bissette, W.H. Busby, Jr.*, W.W. Youngblood*, M. Rossor*, M. Roth*, and J.S. Kizer*. Biol. Sci. Res. Ctr., Univ. North Carolina Sch. Med., Chapel Hill, NC 27514 and MRC Brain Bank, Cambridge, England.

The elucidation of neurochemical alterations in psychiatric and neurologic disease can hopefully result in the development of a rational pharmacotherapy for these disorders. The purpose of the present study was to determine whether alterations in the regional brain concentrations of neurotensin (NT), thyrotropin-releasing hormone (TRH) or somatostatin (SRIF) occur in patients with senile dementia of the Alzheimer type (SDAT). Postmortem brain concentrations of the three peptides were measured by sensitive and specific radioimmunoassay (RIA) methods in three subcortical brain regions (amygdala, nucleus accumbens and posterior hippocampus) and in three cortical regions [Brodmann's areas (BA) 7, 10 and 38] of postmortem samples from ten patients with a diagnosis of senile dementia of the Alzheimer type (SDAT) and ten age- and sex-matched controls (provided by the MRC Brain Bank, Cambridge, England). The diagnosis of SDAT was confirmed histologically. The sensitivity of the RIAs were as follows: NT, 1.25 pg/tube, TRH, 5 pg/tube and SRIF, 1 pg/tube. Protein concentration was determined by the method of Lowry et al. (1951) and data expressed as pg peptide per mg protein. TRH was undetectable in all the cortical brain regions and the posterior hippocampus. No differences in TRH concentrations between the SDAT and control samples were found in the nucleus accumbens and amygdala. The concentration of NT in the amygdala of the SDAT patients was significantly reduced (↓ 30%) when compared to the control group. No differences in NT concentration between the two patient groups were observed in the other five brain regions assayed. As previously described by other research groups, the concentration of SRIF was markedly reduced in the SDAT patients in all of the brain regions studied. The % reductions in SRIF concentration were as follows (region, % reduction when compared to control values): amygdala, ↓ 83%; nucleus accumbens, ↓ 78%; posterior hippocampus, ↓ 92%; BA 7, ↓ 67%; BA 10, ↓ 83%; BA 38, ↓ 83%. These data confirm and extend the previous findings of marked reductions of SRIF-like immunoreactivity in brain regions of patients with SDAT and moreover provide novel data concerning the reduction in NT levels in the amygdala of SDAT patients. The relationship of these findings to the etiology, pathogenesis and clinical presentation of SDAT patients remains unclear.

Supported by NIA AG-03701, NIMH MH-34121 and NICHHD HD-03110.

305.3 CHOLINE ACETYLTRANSFERASE AND MUSCARINIC BINDING IN REGIONS OF NORMAL AND ALZHEIMER'S DISEASED BRAINS. Steven B. Waller, Melvyn J. Ball* and Edythe D. London. NIA, Lab. of Neurosciences, Gerontology Research Center, Baltimore, Maryland 21224: Dept. Pathology, University of Western Ontario, London, Ontario N6A5C1; NIDA Addiction Research Center, Baltimore, Maryland 21224.

Postmortem brain samples were taken from 16 people who died with Alzheimer's disease and 11 control subjects. The following regions were sampled: superior, middle, and inferior temporal gyrus; orbital frontal cortex; middle frontal gyrus; pre- and postcentral gyrus; parietal cortex; calcarine cortex; mammillary body; stria terminalis; hippocampal endplate, H_2, H_1-subiculum; presubiculum; entorhinal cortex; amygdala; and cingulate gyrus. The specific activity of choline acetyltransferase was determined radiometrically using the method of Bull and Oderfeld-Nowak (J. Neurochem., 19: 935, 1971). Estimates of K_D for [H-3] quinuclidinyl benzilate ([H-3]QNB) were obtained from Eadie-Hofstee analysis of saturation isotherm data (n = 5 per group per region). Values of Bmax were calculated from total specific binding at 0.35 nM [H-3]QNB. The percentage of total specific [H-3]QNB binding that was associated with muscarinic agonist high affinity binding sites was determined by an "abbreviated assay" method, as described by McKinney and Coyle (J. Neurosci. 2: 97, 1982). In this assay, specific [H-3]QNB binding was determined in the presence and absence of 20 μM carbamylcholine.

As compared with control samples, choline acetyltransferase was significantly ($p \leq 0.05$) reduced in each Alzheimer's diseased brain region assayed. The reduction in choline acetyltransferase activity ranged from about 50% to 80% in samples of mammillary body and hippocampal endplate respectively. The values of K_D for [H-3]QNB did not differ between Alzheimer's diseased and control brains. Furthermore, there was no evidence for a loss of muscarinic binding or a significant change in the relative proportion of high- to lower affinity binding sites in Alzheimer's disease. (Supported in part by grants from NIH (AG 03047), Med. Res. Council of Canada (PG21) and Ontario Mental Health Foundation (804)).

305.4 CHANGES OF MONOAMINE METABOLISM IN SENILE DEMENTIA OF ALZHEIMER TYPE (SDAT). L.Volicer, L.K.Direnfeld*, P.J.Langlais, M.Freedman*, E.D. Bird*, and M.L. Albert*. E.N.R. Mem. Vet. Hosp., GRECC, Bedford, MA 01730, Boston Vet. Adm. Med. Ctr., Boston, MA 02130, and McLeans Hosp., Waltham, MA 02178.

In order to study the role of monoamines in SDAT, we measured levels of MHPG, HVA, DOPAC, 5-HT and 5-HIAA in cerebrospinal fluid (CSF) of 15 patients with SDAT (66.9 ±1.9 years old) and compared them with levels in 17 control patients (68.3 ±2.0 years old) and in 11 patients with idiopathic Parkinson disease (PD) (67.8 ± 2.2 years old). Both patient groups had mild to moderate forms of their disease. The control group consisted of neurologically intact patients admitted for urological procedures. No subjects had a history of psychiatric illness or alcohol abuse. The SDAT and PD patients had Hachinski ischemic scores less than 4, and no focal abnormality on CT scan or significant EEG abnormal ities. Neither SDAT or control patients were taking any medications known to affect monoamine systems. Dopaminergic medications in PD patients were discontinued 48 hours before lumbar puncture. Lumbar punctures in SDAT and PD patients were performed between 9 and 10 a.m. and the CSF was collected in 1 ml fractions from 6th to 20th ml. In controls the first 3 - 5 ml of CSF were collected at the time of spinal anesthesia. CSF samples were placed on dry ice and stored at -70°C. The CSF levels of MHPG, HVA, DOPAC,5-HT and 5-HIAA were measured in the 6th, 13th and 20th ml from SDAT and PD patients and in an aliquot of control CSF by reversed phase liquid chromatography with electrochemical detection. In the 6th ml of CSF in SDAT patients the level of HVA was higher than in the control aliquot ($p<.02$). DOPAC levels were lower in both SDAT and PD than in controls ($p<.02$). The HVA/DOPAC ratio was higher in SDAT than in PD and control groups ($p<.001$). The HVA levels and HVA/DOPAC ratios were significantly higher in SDAT than in PD in all three fractions. Mean levels of 5-HT and 5-HIAA were similar in all groups. However, there was a significant correlation between 5-HT and 5-HIAA levels in the third CSF fraction of SDAT and PD. This correlation was strongly positive in PD ($r=.965$, $p<.0001$) and negative in SDAT ($r=-.597$, $p<.02$). MHPG levels were similar in SDAT and controls but lower in PD. The HVA/DOPAC ratio reflects the rate of methylation by COMT and its increase in SDAT suggests an increased activity of this enzyme. A positive correlation of 5-HT and 5-HIAA, which we found in PD, was present only in the fraction which most closely reflects cerebral serotoninergic activity. The reversal of this relationship in SDAT indicates differential involvement of the serotoninergic system in these two diseases. (Supported by the Veterans Administration PHS grant CIA-AG00152, and by an award from AFAR).

305.5 HUNTINGTON'S DISEASE: AN IN VITRO RECEPTOR AUTORADIOGRAPHIC STUDY OF NEUROTRANSMITTER RECEPTORS IN THE BASAL GANGLIA AND CORTEX. P. J. Whitehouse*, W.L. Wahl*, S. Folstein*, D. Price and M.J. Kuhar. (SPON: Ky Dismukes). Johns Hopkins Univ., Sch. Med., Dept. Neurosci., Balto, MD 21205.

An in vitro receptor autoradiographic technique was used to map subtypes of muscarinic cholinergic (MC), benzodiazepine (BZ), and GABA receptors in five patients with Huntington's disease (HD; average age 61 yrs, average postmortem delay 7 hrs) and five neurological disease-free matched controls (average age 56 yrs, average postmortem delay 9 hrs). Standard areas of caudate, putamen, globus pallidus, frontal and parietal cortex were dissected and sectioned (8 μm). MC receptors were measured using 1 nM ^{3}H-N-methylscopolamine and 10^{-4} μM carbachol to selectively displace N-methylscopolamine from the high affinity MC sites. BZ receptors were mapped using 1 nM ^{3}H-flunitrazepam and 200 nM CL218,872 to selectively displace flunitrazepam from Type 1 BZ receptor sites. 10 nM ^{3}H-muscimol was used to map GABA receptors with 200 μM GABA used for blanks. The labeled tissue was exposed to LKB tritium-sensitive film. Optical densities were measured using a Gamma Scientific microdensitometer and were converted to receptor concentrations using standard curves.

Significant (Student t-test, $P < .02$) reductions in both total and low affinity MC receptors were found in HD in the caudate, putamen and fairly consistently in deeper layers of both frontal and parietal cortex. Significant ($P < .01$) reductions in total and Type 2 BZ receptors occurred in caudate but not in putamen or cortex. A trend towards increased BZ binding in the globus pallidus was noted. Muscimol binding was significantly ($P < .02$) reduced in both caudate and putamen but not in cortical regions. For the most part these findings are consistent with and extend previous receptor studies in HD. Cholinergic and GABAergic neuronal loss in caudate and putamen may lead to denervation supersensitivity in globus pallidus. Preliminary quantitative analysis of the neuropathological alterations in the individual HD cases did not reveal clear cut relationships between the severity of neuronal loss, degree of gliosis or receptor alterations. Neurotransmitter receptors deserve further study as biological markers for specific populations of neurons affected by neurodegenerative diseases and sites for potential therapeutic intervention.

Supported by MH25951, NS15080, MH00053, NS1637503 and grants from the McKnight, Commonwealth, and Sloan Foundations.

305.6 NORADRENERGIC HYPERACTIVITY IN DEPRESSIVE AND ANXIETY DISORDERS. D.S. Charney* and G.R. Heninger, (SPON: E. Giller). Dept. Psychiatry, Yale Univ. Sch. of Med., New Haven, Ct.

Considerable evidence suggests that anxiety may be associated with increased brain noradrenergic (NA) activity. Pharmacological activation of brain NA neurons produces physiological and behavioral effects in monkeys and humans resembling naturally occuring anxiety states. Yohimbine, an alpha-2 adrenergic autoreceptor antagonist, produces mild anxiety and increases in plasma 3 — methoxy — 4 hydroxyphenylethleneglycol (MHPG) and autonomic symptoms in healthy humans. The purpose of the present investigation was to examine the relationship between drug induced increases in anxiety and noradrenergic function in depressed patients, patients with panic anxiety, and healthy subjects.

Methods: Each patient or healthy subject had two test days within a one week period. Placebo was given on the first test day and yohimbine (20 mg) on the second. Plasma was obtained from blood samples drawn through an indwelling intravenous catheter, both before and at several time points following drug administration. Plasma MHPG was determined by gas chromatography and mass spectrometry. Visual analogue scales and a brief subjective and somatic anxiety scale were used to evaluate changes in behavior and physical symptoms.

Results: In the healthy subjects (N=20), depressed patients (N=25), and anxious patients (N=45) yohimbine caused increases in plasma MHPG which were highly correlated with increases in subject rated anxiety. The depressed and anxious patients experienced significantly more anxiety from the yohimbine than the healthy subjects. In approximately 30% of the depressed patients and 60% of the anxious patients, the anxiety response to yohimbine was greater than that observed in any of the healthy subjects. The plasma MHPG elevation in these patients was markedly greater than that observed in the healthy subjects and in patients who experienced little change in subjective anxiety following yohimbine.

Implications: The finding that yohimbine induced anxiety correlates with MHPG changes in three different samples provides strong support for the hypothesis that NA hyperactivity is involved in the production of some anxiety states. The increased response to yohimbine in a subgroup of depressed patients and a majority of panic anxiety patients indicates that the responsivity of NA systems may be increased in these patients. The results also suggest that the pathophysiology of some forms of anxiety and depression may be similar.

305.7 SEASONAL VARIATION IN PLATELET ^{3}H-IMIPRAMINE BINDING:COMPARABLE VALUES IN CONTROL AND DEPRESSED POPULATIONS. P.M.Whitaker, J.J.Warsh, H.C.Stancer*, E.Persad and C.K.Vint. Clarke Inst. of Psychiatry,Toronto,Ontario, Canada, M5T 1R8.

Recent findings of reduced ^{3}H-imipramine (^{3}H-IMI) binding in depressed patients compared to healthy controls has been proposed as a biological state marker of depression, although two recent studies have failed to replicate this finding.The sites labeled by ^{3}H-IMI are thought to be related to the uptake of serotonin into platelets, a property known to exhibit an annual rhythm. It is possible that ^{3}H-IMI binding also varies throughout the year. If so, such a rhythm should be controlled for in studies of depressed populations as differences in sampling dates of control and depressed populations could result in the observed reduction in ^{3}H-IMI binding to platelets. We report findings here to support this hypothesis.

Subjects studied included four healthy controls (2 male, 2 female) (mean age 30.8 yr.) without current or past psychiatric disorders who gave blood samples (0800-0900 hrs) on the 15th day of each month for one year. Platelet ^{3}H-IMI binding was performed as previously reported (1). Bmax values for ^{3}H-IMI binding decreased from a maximum of 803±88 fmoles/mg. protein in January to a minimum of 207±13 fmoles/mg. in September, returning to the maximum again in December. The affinity (K_D=1.42±0.12 nM) of ^{3}H-IMI binding showed no significant changes over this time interval.

The effect of such a rhythm on comparisons of ^{3}H-IMI binding in normal controls (8 male, 8 female) (mean age 32.6 yr.) and depressed patients (10 unipolar depressed, 7 primary and 3 secondary; 4 bipolar depressed; 2 schizoaffective depressed; mean age 38.6 yr.) was also evaluated. No significant differences were found in Bmax or K_D between normal controls (764±117 and 1.64±0.27, respectively) and depressed patients (798±121 and 1.88±0.38, respectively).

Our results show a highly significant ($p=.001$) seasonal variation in platelet ^{3}H-IMI binding in normal and depressed populations. Furthermore, when annual rhythms are taken into account, there is no difference in the binding parameters in the two populations.

This work is supported by the Ontario Mental Health Foundation.

1. Paul et.al., Arch. Gen. Psych., 38:1315, 1981.

305.8 IMPAIRED SEROTONERGIC FUNCTION IN DEPRESSED PATIENTS: AUGMENTATION BY ANTIDEPRESSANT TREATMENT AND LITHIUM. G.R. HENINGER and D.S.CHARNEY* Dept. of Psychiat., Yale Univ., Sch. of Med., New Haven, CT 06508.

There is considerable data that serotonergic (5HT) function is decreased in depression. Physiologic and behavioral data obtained from animal experiments indicates that long-term antidepressant treatments (ADT) increase postsynaptic 5HT receptor sensitivity. Recently, it has been shown that lithium (LI) produces a rapid antidepressant effect when added to long-term ADT in nonresponding patients. This may involve a facilitation of presynaptic serotonergic function interacting with the ADT induced increase of postsynaptic 5HT sensitivity. There is considerable data indicating that 5HT stimulates prolactin (PRL) release and the 5HT precursor tryptophan (TRP) has been shown to release prolactin in animals and humans. In order to assess whether serotonergic function is decreased in depression and whether ADT and LI increase 5HT function in primates, the PRL response to an intravenous TRP infusion was assessed in depressed patients before and during ADT, and in rhesus monkeys before, during and after LI.

Method: 25 patients and 19 controls each received 7 grams of TRP infused IV over 20 min, and 13 patients were retested on amitriptyline or desipramine treatment. 8 male rhesus monkeys had 200 mg/kg TRP infused over 15 min. before, during and after LI treatment. Plasma obtained before and after the infusion was assayed for PRL using a standard RIA method. The maximal PRL response was measured as the peak post-infusion value minus the pre-infusion baseline.

Results: The maximal PRL response in the patients was more than 2-1/2 times less than the controls, 7 vs 20 ng/ml respectively ($p<.001$). ADT increased the maximal response from 12 to 21 ng/ml ($p<.01$). In rhesus monkeys the maximal response pre-treatment, 2, 7, and 14 days on treatment, and over 21 days post-treatment was 13, 26, 30, 32, and 10 ng/ml respectively. The maximal response during the 3 time periods on LI treatment was different than pre or post LI ($p<.02$).

Implications: The blunted PRL response in patients and its normalization on antidepressant treatment indicates that decreased serotonergic function may be etiologic in depression. The lithium agumentation of the PRL response as early as 2-3 days on LI provides support for the hypothesis that the acute antidepressant effect of LI when added to long-term ADT of non-responding patients involves a facilitation of serotonergic function. These data indicate that the 5HT system plays a major role in the etiology and treatment of depressive illness.

305.9 **CEREBROSPINAL FLUID LEVELS OF NEUROTENSIN-LIKE IMMUNOREACTIVITY IN NORMAL CONTROLS AND IN PATIENTS WITH AFFECTIVE DISORDER, ANOREXIA NERVOSA AND PREMENSTRUAL SYNDROME.** P.J. Manberg*, C.B. Nemeroff, G. Bissette, A.J. Prange, Jr., and R.H. Gerner. (Spon: P. Loosen).
UNC at Chapel Hill, NC, Burroughs Wellcome Co., RTP, NC and VA Medical Center, Long Beach, CA.

Neurotensin (NT), an endogenous tridecapeptide fulfills many criteria to be considered a neurotransmitter (see Nemeroff et al., Handbook of Psychopharmacol. 16:363, 1983). In a previous study (Amer. J. Psychiat. 139:1122-1126, 1982), a subgroup of drug-free acute schizophrenic patients were found to have markedly low CSF NT levels when compared to age- and sex-matched controls. The purpose of the present study was three-fold: (1) to determine whether patients with major affective disorder, anorexia nervosa or premenstrual syndrome have altered CSF NT levels when compared to normal volunteers; (2) to determine whether a concentration gradient of NT exists in CSF and (3) to determine whether probenicid, which inhibits the transport of acidic monoamine metabolites out of CSF, alters CSF NT levels.

Immunoreactive neurotensin (NT) levels were measured in CSF from consenting normal volunteers (n = 13) and patients with premenstrual syndrome (PMS, n = 9), affective disorders (AD, n = 9), or anorexia/bulemia (AB, n = 7) using a modification of a previously described radioimmunoassay (J. Neurochem. 36:1777-1780, 1982). Serial samples from sequential aliquots of CSF (I = 1-12 cc, II = 13-26 cc or III = 27-30 cc) were also obtained to establish the presence or absence of a CSF NT gradient. The samples were assayed without knowledge of the diagnostic identity of the samples. No significant differences between any of the diagnostic categories were present. Mean CSF NT levels (pg/ml; mean ± SEM) were: normals = 241 ± 7; AB = 277 ± 18; PMS = 253 ± 7; AD = 244 ± 13. No correlations between patient age or sex were found and probenicid had no apparent effect on CSF levels of this neuropeptide. Finally, NT levels in each of the CSF aliquots were similar (I = 252 ± 6, II = 246 ± 7, III = 247 ± 10) thereby indicating the absence of a significant CSF concentration gradient. These results confirm and extend prior findings in normals and render specificity to our previous finding of reduced CSF NT levels in a subgroup of unmedicated schizophrenics. (Supported by NIMH MH-34121, MH-33127, MH-22536, and MH-32316, and NICHHD HD-03110).

305.10 **NEUROTRANSMITTER RECEPTORS: THE EFFECTS OF POSTMORTEM DELAY ON A RAT MODEL OF THE HUMAN AUTOPSY PROCESS.** D. Lynch, P. J. Whitehouse and M. J. Kuhar. Johns Hopkins Univ.,School of Medicine, Dept. Neuroscience, Baltimore, MD 21205

Alterations in neurotransmitter receptors have been described in a wide variety of neurological and psychiatric disorders. Receptors are biological markers for neuronal populations which may be specifically effected by the disease process and are also sites for potential therapeutic intervention. Tissue autolysis and other postmortem alterations have been shown to effect certain neurotransmitter system elements, but their effects on receptors have not been systematically examined. We utilized a rat model of the human autopsy process and examined the effects of postmortem intervals (0 - 96 hours) on muscarinic cholinergic, alpha-2, dopamine, and benzodiazepine receptors. After cervical dislocation rat brains were cooled to a final temperature of 20° C or 4° C following the curve of Spokes and Koch (J. Neurochem. 31:381-383, 1978). Using previously published protocols, muscarinic cholinergic, alpha-2 receptors, dopamine, and benzodiazepine receptors were measured in filtration assays using ^{3}H-N-methylscopolamine, para-aminoclonidine, spiperone and flunitrazepam, respectively.

At 20° C N-methylscopolamine binding at death (44.3 ± 2.2 pM per g of tissue) was reduced by 37% at 48 hours and 87% at 96 hours. At 4° C, the loss of receptors was less marked, 21% at 48 hours with no further reduction at 96 hours. Scatchard analysis revealed a decrease in B_{max} as well as some increase in K_D at long postmortem intervals. At 96 hours postmortem delay, an 11% loss of alpha-2 receptors occurred at 4° C, whereas a 97% loss occurred at 20° C. Similar effects were seen with spiperone binding. Flunitrazepam binding studies revealed a puzzling 170% increase in binding when tissue was cooled to 4° C and stored for 48 hours.

We conclude that receptor populations are relatively stable under conditions resembling the normal handling of human autopsy tissue, i.e, storage at 4° rather than 20° and dissection within 24 hrs. Differences in the effect of postmortem delay on different receptor types exist and need to be studied further. Matching of diseased and control patients with regards to postmortem conditions in tissue processing seems clearly warranted in studies of receptor alterations in neurological diseases.

Supported by MH25951, MH00053, DA00266 and grants from the McKnight, Commonwealth, and Sloan Foundations.

305.11 CALCIUM CHANNELS AND CHOLINERGIC RECEPTORS IN SMALL CELL LUNG CARCINOMAS FROM MYASTHENIC SYNDROME PATIENTS. JM Cunningham*, VA Lennon, and EH Lambert. Neuroimmunology and Neurophysiology Laboratories, Mayo Clinic, Rochester, MN 55905.

We have examined small cell carcinomas (SCC) from patients with and without the paraneoplastic Lambert-Eaton myasthenic syndrome (LES) for evidence of voltage-sensitive calcium (Ca) channels and acetylcholine receptors (AChR) LES is a presynaptic neuromuscular disorder characterized by deficient release of acetylcholine (ACh) in response to nerve impulse. Because of evidence that LES is an autoimmune disease (Lang *et al*,Lancet,ii:224,1981;Lennon *et al*, Muscle & Nerve, 5:S21, 1982), and because LES and SCC are strongly associated, we propose that LES in SCC patients might result from an immune response initiated against an antigen common to SCC and cholinergic nerve terminals. Animals immunized with SCC developed antibodies cross-reactive with neuronal cells, but no defect of neuromuscular transmission was detectable by electrophysiologic testing. This result might be explained by a paucity of putative antigen, or suboptimal immunization for that antigen. The putative nerve terminal antigen is probably sparsely represented, as no IgG could be detected bound to LES patient nerve terminals (Engel *et al*,Mayo Clinic Proc.,52:267,1977).

LES IgG was reported to transfer the neurologic defect of LES to mice (Lang *et al*), and freeze fracture studies of neuromuscular junctions of LES patients and mice receiving patient IgG (Fukanaga *et al*,Muscle & Nerve, 5:686,1982;Neurol. 33,S2:157,1983) revealed paucity of active zones and zone particles (presumptive Ca channels). We therefore examined SCC for evidence of Ca channels. Because presynaptic AChR have been implicated in modulation of ACh release, we also tested SCC for muscarinic and nicotonic AChR.

In conditions optimal for binding of a potent voltage-sensitive Ca channel blocker,[^{3}H] nitrendipine, to human and rat brain homogenates, specific binding sites were not consistently demonstrable on crude homogenates or sucrose gradient fractions of SCC. Muscarinic AChR were detected in 2/6 tumors (1 of 4 LES) by specific binding of [^{3}H] quinuclidinyl benzilate. Binding was maximal after 40 min at 37°C and was saturable. Atropine sulfate (10^{-6}M) completely inhibited binding. Scatchard plots for one tumor revealed a K_D of 0.29±0.01nM and maximum receptor density (Bmax) of 52.5± 2.5 fmol/mg protein.The Hill coefficient was 1.09±0.025.No specific binding sites for [^{125}I]α-bungarotoxin were found in Triton X-100 extracts of 4 SCC (3 LES).

These data do not exclude the possibility that Ca channels might be the putative autoantigen of LES. Recent evidence indicates that not all Ca channels are sensitive to dihydropyridines.Ca channels were detected electrophysiologically in 1 SCC cell line(McCann *et al*,Science,212:155,1978). The physiological relevance of muscarinic AChR on SCC is currently being investigated.

305.12 ARE ^{3}H-SPIPERONE AND ^{3}H-DOMPERIDONE LABELLING D_2 DOPAMINE RECEPTORS ON HUMAN LYMPHOCYTES? B.K. Madras, K. Blaschuk*, K. Scully* and S.W. Tang. Psychopharmacology Section, Clarke Institute of Psychiatry, Toronto, Ontario, CANADA M5T 1R8.

^{3}H-Neuroleptic binding to human lymphocytes has been characterized in an effort to identify a peripheral source of brain dopamine receptors (D_2). In addition, several laboratories have recently reported increased ^{3}H-spiperone binding to lymphocytes of schizophrenic patients. (LeFur et al, Life Sci. 32 249 1983, Bondy et al, C.I.N.P. Congress 67, 1982). ^{3}H-Spiperone readily associates with lymphocytes but uncertainty prevails as to whether the binding is to a specific cell surface receptor or represents entrapment within the interior of the lymphocyte. Of several criteria for D_2 receptor identification only saturation of ^{3}H-spiperone binding has been fulfilled in some, but not all investigations. In the present report ^{3}H-spiperone (5nM) and ^{3}H-domperidone (5nM) binding to human lymphocytes was studied, using various experimental conditions. Hank's balanced salt solution was found to be superior to Hepes, or to sucrose HCO_3^-, Ca^{++} medium. Total ^{3}H-spiperone (5nM) binding to lymphocyte cell preparations (10^6 cells/assay tube) was 8869 ± 129 dpm while ^{3}H-domperidone (5nM) binding was lower: 6783 ± 998 dpm (mean ± S.E.M., n=8,4, resp.). Binding of both ligands was displaceable by neuroleptics: The IC_{50} values were obtained using either (+)-butaclamol or haloperidol (10 μM) as baseline. Results are expressed as mean ± S.E.M. of 2-4 separate experiments performed in triplicate.

IC_{50}(nM)

	Lymphocytes		Human Putamen
	^{3}H-spiperone (5nM)	^{3}H-domperidone (5nM)	^{3}H-spiperone (1 nM)
(+)butaclamol	593 ± 201	330 ± 190	14 ± 5
(-)butaclamol	840 ± 140	690 ± 610	10,000
haloperidol	363 ± 19	260 ± 10	18 ± 4
domperidone	1200 ± 300		

These results indicate that D_2 dopamine receptor sites were not detected using either a ligand which penetrates membranes readily (^{3}H-spiperone) or one that may not (^{3}H-domperidone).

Using a simple method of lymphocyte preparation (some platelet contamination) saturable binding of ^{3}H-spiperone (1-5 nM, 10 μM haloperidol, baseline) was obtained in 5 male subjects: Kd range: 4.8-10.3 nM, B_{max} 127-317 fmoles/10^6 cells.

In conclusion, ^{3}H-spiperone and ^{3}H-domperidone do not label D_2 dopamine receptors on human lymphocytes. Because the association of ^{3}H-spiperone with lymphocytes is saturable, neuroleptic binding sites of human lymphocytes can be quantified. Supported by grants from the Ontario Mental Health Foundation and Bickell Foundation.

305.13 RESPONSES OF MUSCLE POSTJUNCTIONAL MEMBRANE AFTER TREATMENT WITH PREDNISOLONE OR NEOSTIGMINE. Angeles B. Ribera and William L. Nastuk. Dept. of Physiology, Columbia Univ., New York, NY 10032

Myasthenia gravis patients under treatment with steroids and anticholinesterase agents sometimes experience an unexpected redevelopment of muscular weakness. Clinically this condition is called "cholinergic crisis". To obtain information which could help explain this condition, we investigated the effect of prednisolone (pred) and neostigmine (neo) on sensitivity and response of the frog muscle postjunctional membrane (PJM) to acetylcholine (ach) and carbamylcholine (carb). One group of frogs (Rana pipiens) received 50 µg/day of pred intraperitoneally for one month, after which time the sartorius muscle was dissected and mounted in a bath of Ringer solution. Non-treated frogs were used for control sartorii. Transmembrane potentials were recorded at junctional regions using conventional techniques. Response of the postjunctional membrane to ach or carb was initially tested by iontophoresis using 1 Hz 50 msec driving pulses applied to high resistance pipettes loaded with 3 M solutions of ach or carb. Positioning of the iontophoretic pipette was adjusted until its tip was located at a responsive spot on the postjunctional membrane as judged by the transient depolarization produced when an iontophoretic current pulse was applied. The iontophoretic pulse strength was then set to give transient PJM depolarization in the 5-10 mV range, and the fiber was allowed to rest for 2-3 min to permit recovery from any receptor desensitization possibly produced during the searching process. For 10 pred treated fibers, PJM depolarization produced during a 20 sec 1 Hz train of 10 msec carb pulses showed a 20-25% decrement. Other pred treated fibers and all control fibers showed no decrement. Also, using a muscle from untreated frogs, after bath application of pred (40 µM to 1 mM), the PJM response to repetitive carb application showed no decrement. We concluded that pred treatment increases the rate of PJM desensitization. In the second part of our study we determined PJM sensitivity to ach and carb before and after bath application of 3 µM neo. For ach, the sensitivity of control fibers averaged 600 mV/nC. It rose to 1250 mV/nC after neo application. For carb, the controls showed a sensitivity of 270 mV/nC and surprisingly the sensitivity increased to 380 mV/nC after neo treatment. For pred treated frogs, the carb sensitivity was 260 mV/nC. It rose to 390 mV/nC after neo application. These results indicate that ach receptor activation is potentiated by neostigmine and that prednisolone treatment can increase the rate of desensitization. Myasthenic patients undergoing steroid and anticholinesterase therapy may be susceptible to transmitter overload at neuromuscular junctions.

Supported by a Myasthenia Gravis Foundation grant to W.L.N.

COLOCALIZATION OF TRANSMITTERS

306.1 β-ENDORPHIN 28-31 MODULATES BEHAVIORAL ACTIONS OF α-MSH. M.D. Hirsch* and T.L. O'Donohue (SPON: E. Cantor). ETB, NINCDS, NIH, Bethesda, MD 20205.

Recently, many neurons have been demonstrated to secrete multiple neurotransmitters. One of the first multiple neurotransmitter systems demonstrated is the opiomelanotropinergic system which secretes alpha-melanocyte stimulating hormone (α-MSH) and beta-endorphin (βE) from endocrine cells in the intermediate lobe of the pituitary and neurons in the central nervous system. It is of particular interest to determine how neurotransmitters in a multiple neurotransmitter neuron interact. Previous studies have shown that the non-opioid C-terminus of βE modulates α-MSH actions at melanophore receptors (cf. Logan, A. et al., Peptides, 2: 121, 1981). In this study, we investigated the pharmacological interactions between the βE fragment, βE 28-31, and α-MSH.

Adult, male Sprague-Dawley rats (250-350g) received intracerebroventricular (ICV) administration of peptide solutions containing 0.1-50µg of α-MSH in the presence or absence of 0.01-50µg of βE 28-31. Control solutions contained saline vehicle in place of respective peptides. All solutions were injected in volumes of 1-20µl. Grooming activity was quantitated over a 55 min. period as described previously (Gispen, W.H. et al., Life Sci. 17: 645, 1975). Excessive grooming has been interpreted as indicative of an increased state of arousal.

The results indicated that βE 28-31 antagonizes α-MSH-induced excessive grooming in rats in dose-related fashion. In addition, βE 28-31 appears to act like a classical competitive antagonist since it effected a 5-fold increase in the median effective dose (ED50) of α-MSH without apparently influencing maximum dose effects of the latter peptide. βE 28-31 (10µg ICV) also significantly reduced ($p < .001$) the stretch-yawn syndrome which followed bouts of grooming induced by α-MSH (5µg ICV). The results of this study demonstrate that βE 28-31, the C-terminal tetrapeptide of βE, can modulate the central actions of α-MSH. This finding suggests that peptides secreted from a single neuron can have interactive and antagonistic actions.

306.2 **DIFFERENTIAL RELEASE OF CATECHOLAMINES AND ENKEPHALIN-LIKE PEPTIDES FROM THE BOVINE ADRENAL GLAND,** **B.A. Barron* and T.D. Hexum (SPON: A. Earle). Dept. of Pharmacology, Univ. Neb. Med. Ctr., Omaha, NE 68105.**

Norepinephrine (NE), epinephrine (E) and met^5-enkephalin immunoreactive material (ME-IRM) have been shown to have important effects on cardiovascular function after either peripheral or central administration. These catecholamines and ME-IRM are stored in chromaffin granules of the adrenal medulla and can be released by splanchnic nerve stimulation. The relationship between the release of catecholamines and ME-IRM has not been described. Secretion of CA and ME-IRM from the bovine adrenal gland was studied following stimulation by a number of agents. The substances used included nicotinic and muscarinic agonists and antagonists, (ACh, 1,1-dimethyl-4-phenylpiperizinium -DMPP, nicotine, atropine, hexamethonium) and the opioid agonist, etorphine. The isolated glands were retrogradely perfused with Lockes solution at 2 ml/min, drugs were infused by syringe pump at 0.02 ml/min. The perfusate was collected on ice for 3 min periods. Stimulation by ACh, DMPP or nicotine produced a dose dependent increase in the release of both CA and ME-IRM as measured by HPLC with electrochemical detection and radioimmunoassay, respectively. These cholinergic agonists produced a greater increase in the release of NE than of E. The ratio of NE/E released increased from a baseline value averaging 0.6 to as high as 2.0. Atropine reversed the increase in NE/E seen with ACh without significantly decreasing the stimulated release of total catecholamines. Atropine did not have this effect on the DMPP-induced release. Etorphine also attenuated the increase in NE/E seen with ACh but to a lesser extent than atropine. Etorphine decreased the total amount of catecholamines released. The ratio of ME-IRM/CA remained the same for all agonists used. These results suggest the presence of muscarinic and opioid modulation of secretion by the bovine adrenal gland.

This research was supported by the American Heart Association, Nebraska Affiliate, and the Department of Health, State of Nebraska.

306.3 RELATIONSHIP BETWEEN ENKEPHALINS, CHOLECYSTOKININS AND OXYTOCIN OR VASOPRESSIN IN MAGNOCELLULAR HYPOTHALAMO-HYPOPHYSEAL NEUROSECRETORY NEURONS.
J.J. Vanderhaeghen+, P. Verbanck*+, C. Deschepper*+, F. Lotstra*+, D.R. Liston*++ and J. Rossier++.
+: Brugmann University Hospital. Neuropathology Neuropeptide Research Laboratory, Free University of Brussels, Belgium. ++: Laboratoire de Physiologie Nerveuse, Centre National de la Recherche Scientifique, Gif sur Yvette, France.

Using radioimmunoassay the cholecystokinin immunoreactivity (CCK-IR) of the rat pituitary posterointermediate lobe is significantly higher in males and decreases after salt loading in either sex or in males 21 days after either castration or daily subcutaneous oestradiol injections. Using immunocytochemistry, CCK-IR disappears from the external median eminence selectively 21 days after adrenalectomy.

Oxytocin, Met-enkephalin, bovine adrenal Pro-enkephalin A and Cholecystokinin immunoreactivities were studied using immunocytochemistry. In bovine they are present sometimes coexisting in the same cell, in the magnocellular neuronal cell bodies of the dorsal part of the supraoptic nucleus and of the peripheral part of the paraventricular nucleus and in neuronal terminals of the external part of the median eminence and of the posterior hypophysis. Quite the contrary Leu-enkephalin immunoreactive neuronal cell bodies are located in the ventral part of the supra-optic and in the central part of the paraventricular nuclei similarly to Vasopressin.

Those results show that sex and factors affecting Vasopressin and/or Oxytocin in the posterior pituitary and the external median eminence may also affect sometimes differently CCK-IR and that a neuronal system containing Oxytocin, Met-enkephalin and Cholecystokinin exists separately from the system already reported in the rat containing Vasopressin, Neoendorphin and Dynorphin in the hypothalamus. They will be discuss in relation to functional significance of co-localization.

This work was supported by Funds of FNRS 81-83, FRSM 3.4521.82-85, Rotary ANAH 1982 and Medical Queen Elisabeth Foundation 81-83.

306.4 PRESENCE OF TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN RAT BRAIN SEROTONERGIC NEURONS. Towle, A.C.*, Joh, T.H., Lauder, J.M. (SPON: H. Krebs) Anatomy Dept., UNC Med. Sch., Chapel Hill, NC 27514; Dept. Neurol., Cornell Univ. Med. Coll., New York, NY 10021.

Serotonergic immunoreactive (5HT-IR) neurons, notably in the pons-medulla, react with an antiserum directed against purified tyrosine hydroxylase from bovine adrenal medulla. Rats were perfused with 4% paraformaldehyde, 70mM phosphate, pH 7.2 and the brain was prepared for routine paraffin embedding. Adjacent 10 micron sections were rehydrated, pretreated with trypsin (1.2mg/ml, 3 min), incubated with primary antisera (1:1000) and then visualized with a biotinylated second antibody-avidin-biotinylated peroxidase complex (ABC) method. All tyrosine hydroxylase immunoreactivity (TH-IR) was blocked by preabsorption with 1µg/ml TH and the 5HT-IR by preabsorption with 2µM 6-OH-tetrahydro-ß-carboline.

Many darkly stained TH-IR neurons were seen in the catecholamine cell groups which have been described. Interestingly, many lightly stained cells were also observed in the ventral pons-medulla. When compared to 5HT-IR neurons in adjacent sections, evidently the lightly stained TH-IR neurons have a similar size, shape and distribution. Moreover, the lightly stained TH-IR cells are exclusively located in regions that contain 5HT-IR neurons. In a previous report, we have shown that >85% of brain serotonergic neurons are permanently eliminated by neonatal intracisternal 5,7 DHT & DMI treatment, while norepinephrine content is unchanged. Under such conditions, the lightly staining TH-IR neurons were absent as well, yet, the TH staining of catecholamine neurons, such as locus coeruleus and substantia nigra appeared unchanged. To further demonstrate that serotonergic neurons contain TH-IR, we sought to double-stain these cells by taking advantage of the fact that the cell nucleus reacts only with the serotonin antiserum. Using a rat anti-5HT and a rabbit anti-TH, it was possible to demonstrate immuno-staining for both antigens in the same neuron.

Such observations suggest that this TH antiserum can bind to a protein in serotonergic neurons, although the identity of this protein is not certain. Since tyrosine hydroxylase and tryptophan hydroxylase share many similarities, including common cofactor requirements, it is possible that a component of the TH antiserum may cross-react with both enzymes. In addition, cells in the pineal gland, which have high levels of serotonin produced via a non-neural form of tryptophan hydroxylase, do not show any TH-IR. On the other hand, serotonergic neurons may synthesize TH constitutively, allowing for low levels of TH-IR to be detected.

NS-15706, NS-07166, NS00507.

306.5 LOCALIZATION OF AROMATIC L-AMINO ACID DECARBOXYLASE AND VASOPRESSIN IN NEURONS OF THE SUPRACHIASMATIC NUCLEUS OF RATS OF THE SPRAGUE-DAWLEY AND BRATTLEBORO STRAINS. C.B. Jaeger, V.R. Albert, T.H. Joh and D.J. Reis. Laboratory of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

We have recently observed a population of neurons in the suprachiasmatic nucleus (SCN) of rat hypothalamus that contain the enzyme aromatic L-amino acid decarboxylase (AADC). Since some neurons of SCN store arginine vasopressin (AVP), we sought to determine whether: (a) AADC and AVP co-exist in the same neurons of SCN; (b) AVP neurons in magnocellular regions of paraventricular and supraoptic nuclei also contain AADC; (c) if rats of the Brattleboro strain which fail to express AVP also contain AADC in neurons of SCN.

Rats were perfused transcardially and their brains processed for immunocytochemistry using the PAP technique of Sternberger. Antibodies to AADC and tyrosine hydroxylase (TH) were raised against enzymes from bovine adrenal medulla. Distribution of AADC, TH or AVP was mapped in the SCN on adjacent sections. In addition, sequential co-localization, without antibody complex removal (Sternberger and Joseph, J. Histochem. Cytochem., 27:1424, 1979), of AADC and AVP were studied in the same section.

AADC was localized in small densely packed neurons of the rostral and caudal poles as well as in parvocellular neurons of the mediodorsal and dorsolateral segments of SCN. These neurons did not contain TH. Some TH positive neurons also containing AADC were found in cell poor regions surrounding the SCN and in the periventricular nucleus of the hypothalamus. In contrast, AADC was not seen in magnocellular neurons of the supraoptic or paraventricular nuclei. A striking overlap was observed of the distribution pattern of AVP positive and AADC immunoreactive cells in the SCN. The coexistence of AVP and AADC was confirmed by double staining. The distribution of AADC positive neurons in the SCN of homozygous Brattleboro rats was similar to that of the Sprague-Dawley strain. We conclude: (a) that neurons of the SCN contain AADC but probably not monoamines; (b) that in these neurons, AVP and AADC are co-expressed; (c) that the expression of AADC and AVP is not linked; (d) that a subpopulation of of AVP-containing neurons will express AADC. The substrate for, and product of, the decarboxylation reaction in SCN is unknown. (Supported by NIH Grants HL 07379 and HL 18974.)

306.6 OCCURRENCE AND ACTIONS OF VASOPRESSIN IN MAMMALIAN SYMPATHETIC GANGLIA. M.R.Hanley, H.P.Benton*, S.Lightman*, C.J.Kirk*, and R.H.Michell*. Dept. of Biochemistry, Imperial College, London; CRC Cell Proliferation Unit, Royal Postgraduate Medical School, London; Dept. of Medicine, Westminster Medical School, London; Dept. of Biochemistry, University of Birmingham, U.K.

Extracts of rat superior cervical ganglion (SCG) contain vasopressin-like immunoreactivity (VLI) in concentrations higher than that at any site outside the neurohypophysis. The vasopressin content was measured by radioimmunoassay using an antibody to vasopressin with less than 0.1% cross-reactivity with oxytocin. Examination of extracts from parasympathetic (e.g. submandibular) and sensory ganglia did not show high levels of VLI. HPLC analysis of sympathetic ganglion extracts exhibited a single immunoreactive peak co-eluting with synthetic arginine-vasopressin.

VLI was localised in rat SCG using immunofluorescence on slide-mounted, formaldehyde-fixed cryostat sections. Antisera to both vasopressin and oxytocin stained large neurones of the ganglion; however the staining was selectively abolished by pre-treatment with vasopressin only. In double staining experiments or staining of serial sections, the VLI-positive neurones appeared to be the same population staining positively for the noradrenergic marker dopamine-ß-hydroxylase (DBH). Indeed, in the rat and monkey SCG all of the DBH-positive neurones were positive for VLI. Within the ganglion, a plexus of nerve fibres staining positively for DBH also stained positively for VLI.

Activation of V_1-vasopressin receptors in liver and artery is associated with calcium mobilisation and the stimulation of inositol lipid turnover. Rat SCG were pre-labelled with ^{3}H-inositol, and then stimulated with vasopressin. Within 15 sec., there was a large accumulation of the initial products of inositol lipid breakdown. The magnitude of the vasopressin-stimulated response was manyfold larger than the response of these ganglia to muscarinic stimulation. The inositol lipid response was half-maximal at 30 nM vasopressin, was not stimulated by oxytocin, and was inhibited by a selective V_1-receptor antagonist.

Examination of rat coeliac ganglion by both radioimmunoassay and immunofluorescence demonstrated a similar vasopressin content and identical localisation pattern to that described in the rat SCG. Moreover, examination of the SCG of mouse, guinea pig, and monkey by the same techniques have shown the same co-localisation of VLI to DBH-positive sympathetic neurones.

The possible occurrence of VLI in the peripheral projections of sympathetic neurones was examined in several sympathetically-innervated tissues. Numerous VLI-positive fibres were detected interposed between the smooth muscle layers of blood vessel walls, in the hilus and cortex of the kidney, and in the hilar region of the liver.

307.1 BILATERAL CRYOGENIC BLOCKADE OF THE AMYGDALOID CENTRAL NUCLEI WILL PREVENT VENTRICULAR FIBRILLATION IN THE ISCHEMIC HEART OF THE PSYCHOLOGICALLY STRESSED PIG. J. E. Skinner, M-F. Montaron* and C. M. Pratt*. Neurophysiology Section, Neurology Department and Neuroscience Program, Baylor Col. of Med., Houston, TX 77030

Previous studies in our laboratory have shown that psychologic stressors (e. g., an unfamiliar environment or mild cutaneous shock) must be present for acute coronary artery occlusion to result in ventricular fibrillation (VF) (Skinner, Lie and Entman, Circulation, 1975). Bilateral cryogenic blockade in the trajectory of the frontocortical-brainstem pathway will prevent VF in the ischemic heart of the psychologically stressed pig (Skinner and Reed, Am. J. Physiol., 1981). The amygdaloid nuclei send projections through the diagonal band and stria terminalis to the same hypothalamic brainstem regions that receive collateral inputs from the frontocortical-brainstem pathway. Thus, a component of the descending deleterious projections of the brain to the heart during psychologic stress could arise from the amygdaloid nuclei. We now report that in 4 out of 4 pigs tested, bilateral cryogenic blockade (-10°C) arising from cryoprobes placed in the anterior portion of the central amygdaloid nuclei, will indeed prevent VF following acute left anterior descending coronary occlusion in pigs stressed by an unfamiliar environment. (Supported by HL17907-07 and HL28425-01).

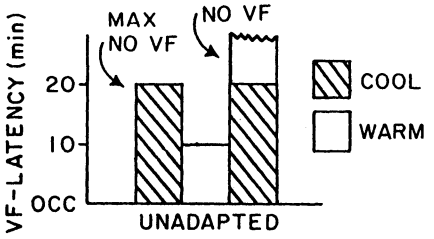


Figure 1.: Experiment in a typical pig. During cryogenic blockade of the amygdaloid nuclei (shaded), coronary artery occlusion did not result in ventricular fibrillation (VF) within the maximum period of reversible ischemia (MAX). The artery was then released and the heart allowed to recover. The next day VF occurred during the expected 9-14 min interval. The artery was then released, the VF electroconverted and the heart once again allowed to recover. On the third day cryogenic blockade was maintained for 20 min following arterial occlusion, and then turned off. In this case VF never did occur, even though a permanent myocardial infarction was created.

307.2 EFFECTS OF FASTIGIAL NUCLEUS STIMULATION ON BEHAVIOR AND CARDIOVASCULAR PARAMETERS IN THE FREE-MOVING DOG. J. T. Braggio, and K. J. Dormer. Univ. of Okla. Health Sciences Center, Okla. City, OK 73190.

The purpose of the present experiment was to investigate the effects of mono- and bi-polar stimulation to the fastigial nucleus (FN) of the cerebellum in free moving dogs. Aseptic surgical procedures were used to implant FN electrodes and cardiovascular (CV) instrumentation in dogs using techniques previously described by Dormer and Stone (1976). The FN electrodes were chronically implanted in rostromedial FN to elicit the greatest changes in heart rate (HR), and arterial pressure (AP). With a left thoractomy, solid state pressure transducers were placed in the descending aorta. All wires exited in the neck region, except for the pressure transducers which contained a percutaneous connector plug in some dogs. Once each dog had recovered and adjusted to the laboratory (2-3 weeks), AP readings were recorded over multiple 40 min. sessions, during which time the FN was stimulated by a programmable stimulator carried in a vest worn by the dog. Stimulation was continued for 15-20 sessions while AP, HR, and behavior were recorded. Stimulus parameters consisted of a charge-balanced, biphasic rectangular waveform, 100 pulses/sec., 100-500 μA with an 11 min. duty cycle, which was delivered both either to the mono- or bi-polar electrodes. It was found that when FN stimulation of 400 μA was used the dogs tested showed an increase in the frequency of swallowing, licking, shaking, loss of balance, raising rear and front paws upwards, genital groom, whining, and tail wag. Even at above threshold values of FN stimulation we did not observe rage or attack behavior in the dogs tested in this study. It was also found that FN stimulation of 400 μA produced increases in blood pressure which preceded, by about 1 sec., the behaviors described above. These data support the results of Berntson and Paulucci (1979), Reis, Doba, and Nathan (1973), and Dormer and Stone (1982) since all of these investigators have also described the presence of grooming and "anxious" whining behaviors. These results suggest that the cerebellum may function as a somato-visceral integrator, or possibly a fine tuner of CV parameters during exercise (Dormer and Stone, 1982), and at reduced levels of activity in the free moving dog. In summary, the cardiovascular and behavioral changes which are observed following FN stimulation may reflect an integrated response pattern which may be influenced by other structures in the brain, such as the nucleus tractus solitarius. (Supported by NIH grant HL24082).

307.3 BEHAVIORAL RESPONSES TO LOCUS COERULEUS STIMULATION IN FREELY MOVING CAGED MONKEYS. D.E. Redmond, Jr., Y.H. Huang, S.J. Grant. Neurobehavior Lab., Yale Univ., New Haven, Connecticut 06510, U.S.A.

Effects of lesions and electrical field stimulation in monkeys suggested a role for the noradrenergic locus coeruleus (LC) as part of a brain "alarm" system involved in fear, anxiety, and opiate withdrawal (Redmond et al, Brain Res. 116:502, 1976; Redmond, Animal Models in Psychiatry and Neurology, pp. 293-306, 1977). Although effects of electrical or pharmacological activation of the LC were similar to induced fear and to opiate withdrawal effects during chair-restraint, they are difficult to compare with behaviors of unrestrained monkeys. The present study was therefore undertaken to examine the behavioral effects of electrical stimulation of the LC in awake, socially interacting, cage-restrained Macaca arctoides. Bipolar electrodes were implanted stereotaxically and LC placements confirmed histologically. Biphasic electrical square waves (ranging from .125-1.5 mAmp intensity, 0.5 msec duration, 30 Hz) were delivered for 1-10 seconds via cable and commutator or via radiotelestimulation to a total of 7 monkeys. Videotaped data were quantitated by two raters who were uninvolved in the original experiments. Defined movement sequences were scored for one-second periods before, during, and after stimulation.

Above a threshold level for each animal, behavioral effects were stimulus-intensity dependent over the range studied. The lowest level stimulation interrupted and/or prevented eating in food deprived monkeys during and immediately after the stimulation without other observable effects (Leverenz et al, Neurosci. Abst. 4:177, 1978). The next higher intensities produced an alerting response characterized by interruption of on-going behavioral activity and visual scanning of the environment. Higher levels of stimulation elicited some chewing, yawning, scratching, and grasping, all prominent behaviors seen in chair-restrained monkeys. In addition pacing, running, jumping up to a perch, and attempting to escape from the cage were seen at these levels in some monkeys. There were no signs of pain, e.g. no grimacing, submissive lip smacking, or screeching. Representative sequences of stimulation recorded on 16 mm. color film will be shown.

Since all of the reported behaviors occur in cage restrained monkeys during mild anxiety or acute fear and during naloxone precipitated morphine withdrawal, these findings are consistent with neuroanatomical, pharmacological, and neurophysiological data and previous studies which have associated locus coeruleus function with alarm, fear, and opiate withdrawal.

Supported in part by MH31176, DA02321, the H.F.Guggenheim Foundation, and R.S.C.D.A. DA00075 to D.E.R.

307.4 HEART RATE SPECTRAL ANALYSIS ALLOWS NON-INVASIVE QUANTIFICATION OF AUTONOMIC FUNCTION IN HEALTHY AND HYPERTENSIVE MAN. B.H. Pomeranz, R.B. Macaulay*, M.A. Caudill*, I. Kutz*, D. Adam*, R.J. Cohen*, A.C. Barger*, D.C. Shannon* and H. Benson*. Dept. of Zool. and Physiol., Univ. of Toronto, Toronto M5S 1A1, Dept. of Med. and Physiol., Harvard Med. Sch., Boston 02215, Harvard-MIT Div. of Health Sciences, MIT, Cambridge 02139.

The autonomic nervous system mediates fluctuations in heart rate. We report that spectral analysis of spontaneous heart rate fluctuations provides a non-invasive means of assessing autonomic function in normal and hypertensive man. Fluctuations below 0.15 Hz are increased by standing, and are jointly mediated by the sympathetic and parasympathetic nervous systems. In contrast, higher frequency fluctuations are decreased by standing and are parasympathetically mediated. Finally hypertensive subjects demonstrate marked reduction of low frequency fluctuations implying abnormalities in autonomic function with elevated blood pressures.

307.5 PITUITARY BETA-ENDORPHIN SECRETION FOLLOWING THE ADMINISTRATION OF OPIATE- AND BENZODIAZEPINE-RECEPTOR ACTING DRUGS. G.P. Mueller and S. Maiewski*. Department of Physiology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814

The ability of physical stimuli to evoke the release of pituitary immunoreactive beta-endorphin (iβ-END) is well documented. Further, recent findings in rats indicate that the magnitude of the plasma iβ-END response may be directly related to the degree of stress experienced (Mueller, G.P., Life Sci., 29:1669, 1980). Therefore, the mechanisms through which physically stressful stimuli evoke iβ-END release are probably separate and additive. Depending upon their nature, physically stressful stimuli can cause pain and also result in increased levels of perceived anxiety; both experiences may contribute to the generation of a pituitary iβ-END response. Accordingly, we have used a pharmacologic approach to evaluate the participation of an opiate-receptor mechanism (pain) and a benzodiazepine-receptor mechanism (anxiety) in mediating the release of pituitary iβ-END. Plasma levels of iβ-END were measured by RIA using antiserum raised against camel $\beta\text{-END}_{1-31}$. The RIA recognizes human β-lipotropin and β-END equally but does not detect N-terminal fragments of β-END (enkephalin and α-endorphin). Basal levels of circulating iβ-END were not significantly altered by either morphine (5 mg/kg) or diazepam (5 mg/kg; ip; 30 min) given in doses which have been shown to be effective in modifying behavioral responses. Further, neither drug significantly altered the 7-fold increase in plasma iβ-END evoked by physical immobilization. Both drugs did, however, tend to reduce the magnitude of the plasma iβ-END response to stress. In a second experiment, morphine and diazepam were administered alone and in combination. Again, given alone each drug tended to reduce the amount of iβ-END released in response to stress and this tendency became significant following combination treatment. Acute administration of β-carboline-3-carboxylic acid ethyl ester (β-CCE; 2 mg/kg; ip), an active antagonist of benzodiazepine receptors did cause a modest 2-fold rise in basal levels of plasma iβ-END (364±83 pg/ml vs. 833±220 pg/ml; $P<0.50$) whereas, the inactive antagonist ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4-imadazo-[1,5-A][1,4] benzodiazepine-3-carboxylate (RO-15-1788; 10 mg/kg,ip) was without effect. Taken together, these findings indicate that opiate- and benzodiazepine-receptor mechanisms do not profoundly influence the release of pituitary iβ-END under the conditions described here. This suggests that the unaltered, conscious perception of pain or anxiety are not the only components in the mechanism(s) which evoke the release of pituitary iβ-END in response to physical stress.

307.6 ROLE OF VASOPRESSIN IN STRESS-INDUCED INHIBITION OF TESTICULAR STEROIDOGENESIS. R. Collu, W. Gibb* and J. R. Ducharme*. Res. Unit on Reprod. and Develop. Biology, Ped. Res. Centre, Ste-Justine Hosp. and Université de Montréal, Montréal, Québec H3T 1C5.

Immobilization, a form of psychological stress, rapidly induces in rats a state of hypogonadism characterized by low circulating levels of testosterone (T) and by testicular hyposensitivity to gonadotropins (Biol. Reprod. 27: 616, 1982). The latter appears to be due to a post-cyclic AMP inhibition of T biosynthesis (Endocrinology 109: 1254,1981). Recently, Adashi and Hsueh have reported that arginine-vasopressin (AVP) is capable of exerting a direct inhibition of testicular T biosynthesis in vitro (Endocrinology 109: 1793, 1981). Although the release of AVP during stress is not firmly established, it appears to occur under special circumstances (Endocrinology 104: 641, 1979). We decided, therefore, to verify whether the lack of AVP, which occurs in Brattleboro rats, might modify the testicular response to stress. Groups of adult male homozygous Brattleboro rats with diabetes insipidus (DI rats) and of normal male rats of the same Long Evans strain (LE rats) were immobilized for 2 or 3 h by taping the four limbs or were left undisturbed as controls. At the end of the stressing period the animals were decapitated jointly with unstressed controls. Trunk blood was collected for T and LH determination by specific radioimmunoassay. The testes were removed and treated as previously described to obtain a purified preparation of Leydig cells. These were incubated for 3 h in multiwell dishes at a concentration of 5×10^5 cells/ml of incubation medium (Ham's F12 and DME with salts and antibiotics) with or without hCG 0.1, 1.0, 10 mIU or Bromo-cyclic AMP (cAMP) 1 mM. Three replicate dishes were run for each point and the experiments were repeated two to six times. At the end of the incubation period, media were collected and stored at -20°C until T determination. hCG induced a significant dose-dependent increase of in vitro T production by Leydig cells obtained from control LE and DI animals. Immobilization for 2 or 3 h resulted in a decrease of plasma T levels in LE rats and reduced unstimulated and hCG- and cAMP-stimulated release of T by purified Leydig cells. A 2 h stress failed to reduce plasma T levels in DI rats as well as unstimulated and stimulated in vitro T production. However, results obtained with 3 h-immobilized DI rats were similar to those obtained with stressed LE animals. These results confirm previously obtained data showing that immobilization stress can rapidly induce a state of post-cAMP inhibition of T biosynthesis. They indicate, furthermore, that AVP may intervene, at least initially, in such phenomenon either directly at the testicular level or through the hypothalamic-pituitary axis.

DEVELOPMENTAL SPECIFICITY

308.1 PROPERTIES OF A SPECIFIC TRANSMITTER MEDIATED CONNECTION FORMED BETWEEN IDENTIFIED NEURONS OF APLYSIA IN DISSOCIATED CELL CULTURE. S. Schacher* and J. S. Camardo* (SPON: S. Rayport). Center for Neurobiology and Behavior, Columbia University, College of Physicians & Surgeons, and New York State Psychiatric Institute, New York, N. Y. 10032.

Identified neurons isolated from the abdominal ganglion of juvenile Aplysia can regenerate neuritic processes and reestablish chemical connections in dissociated cell culture. We have recently used this culture system to examine the development of chemical connections between the identified cholinergic neuron L10 and one group of its follower cells, the Left Upper Quadrant Cells (L2-L6, LUQ cells). Our experiments addressed two questions. First, will L10 form specific synapses? Second, to what extent do the in vitro connections resemble the synapses in vivo?

L10 was cultured with both LUQ cells and Right Upper Quadrant Cells (RUQ cells), a group of non-target cells which make cholinergic receptors. After 3-6 days, L10 elicited an IPSP onto the LUQ cells in five of six cultures. In contrast, L10 did not elicit a response in the RUQ cells, despite the fact that the RUQ cells elaborated neurites which overlapped those of L10 and responded to iontophoresis of acetylcholine (ACh). L10 in culture therefore will select its usual target from a group of neighboring cells.

We next examined the properties of the L10-LUQ connections formed in 3-6 day cultures in which L10 was presented with LUQ cells alone. In these cultures, L10 elicits a dual fast-slow inhibitory IPSP. The fast IPSP has a latency of 20 msec, is sensitive to intracellular chloride, and reverses with hyperpolarization. The slow IPSP is elicited by repetitive firing of L10, is insensitive to intracellular chloride, and does not reverse with hyperpolarization. ACh iontophoresis onto the cell body and regenerated processes of the LUQ cells elicited both responses.

Further examination of this synaptic connection has shown that the fast IPSP decreases in size with stimulation greater than 3 Hz and shows no post-tetanic potentiation (PTP) with the same frequency tetanus for 10-30 seconds. Ohmori et al. (Science, 213:1016) have shown that PTP develops gradually at this synapse through the juvenile life of the animal, long after the connection itself is formed, and suggested that the plastic functions of the synapse are acquired as a separate stage in synapse maturation. Since PTP has developed at this synapse in animals from which these cells were cultured, our observation suggests that the maturation of this synapse in vitro is not complete.

We conclude that: (1) The identified neuron L10 will form synapses selectively with its usual target cells, and will avoid forming synapses with non-target cells. (2) The synapses established in short-term culture are indistinguishable from those in vivo under appropriate culture conditions, with the important exception that they show no PTP.

308.2 PRENATAL DEVELOPMENT OF CALLOSAL AND INTRAHEMISPHERIC CORTICO-CORTICAL INPUT TO PREFRONTAL CORTEX IN THE RHESUS MONKEY. M.L. Schwartz and P.S. Goldman-Rakic, Sec. Neuroanat., Yale University School of Medicine, New Haven, CT 06510

Neocortical neurons in the rhesus monkey are generated by embryonic day 100 (E100) and are in situ shortly thereafter (Rakic, 75). Little is known, however, about the development of connections between cortical regions during the prenatal period. Here we use HRP and fluorescent dyes to examine the callosal and associational projections to the prefrontal cortex from E100 on. HRP crystals were deposited using fine micropipettes to restrict the spread of enzyme in immature cortex. Animals were injected in the cortex surrounding the principal sulcus (PS) at E104, E124, E131, E133 and E137. Golgi impregnated material provided data on the morphological maturity of neurons at these ages.

Tissue from the E104 fetus is currently being analyzed. By E124, labeled neurons are observed in all cortical regions projecting to the PS in mature monkeys, i.e., the cingulate cortices, superior temporal gyrus and posterior parietal cortex. Contralaterally, the heaviest labeling is found in the PS, arcuate cortex and anterior cingulate. Both callosal and associational neurons were found in the lower two thirds of layer III and to a lesser extent in infragranular layers. The layer III neurons were pyramidal while labeled cells in infragranular layers had varied morphologies. The density of callosal neurons exceeds that of associational neurons. Tangential variation in density gradually emerges over the period examined. At early ages neurons are elongated and only lightly labeled. With increasing age they assume a more mature appearance but are always easily distinguished from those seen in monkeys injected as adults. Examination of Golgi stained material indicated that by E104 many neurons have apical dendrites which reach the superficial portion of the developing cortex. In contrast, the basilar dendrites and branches of apical dendrites are short and few in number. No dendritic spines are evident. These dendritic systems gradually emerge over the prenatal period examined, although dendritic spines are still infrequent as late as E140.

Our results reveal that cortical regions which project to the prefrontal cortex in adults already have connections with this area by E124. However, maturation of dendritic arbors continues long after the axons of layer III cells have reached their targets. Further, the lack of dendritic spines on these cells indicates that much of this maturation occurs in the absence of a large portion of their afferent input. Supported by MH00298, MH38546 and MH08308.

308.3 A POSITIONAL BASIS FOR SELECTIVE REINNERVATION OF ADULT RAT MUSCLES. D.J. Wigston and J.R. Sanes. Dept. of Physiology, Washington University School of Medicine, St. Louis, MO. 63110.

We report two extensions of an experiment (Wigston and Sanes, Nature 299: 464, 1982) which showed that adult mammalian muscles can be selectively reinnervated. As before, we transplanted pieces of external intercostal muscle from different segmental levels to the neck of adult rats, removed the superior cervical ganglion, and attached the proximal cut end of the sympathetic trunk to the surface of the transplant. Preganglionic axons in the trunk reinnervated the muscle. After 3-5 wks, we determined the segmental origin of the innervation by stimulating each thoracic (T) ventral root that contributes axons to the trunk (T1-T6), while recording intracellularly from muscle fibers in the transplants.

Previously, we showed that intercostals transplanted from T2 and T8 levels are differentially reinnervated by preganglionic axons (op. cit.). T2 muscles receive more inputs from axons in rostral ventral roots (T1 and T2) but fewer from caudal roots (T4-T6) than T8 muscles. To test the idea that such selectivity has a positional basis, we transplanted T3, T4, and T5 intercostals in a new series of experiments, and assessed their reinnervation. T3, T4, and T5 muscles (n=10 each) were all reinnervated to a comparable extent. However, the average segmental origin of inputs to transplants from different levels differed systematically: it was most rostral to T3 muscles, intermediate to T4 muscles, and most caudal to T5 muscles. Similar results were obtained whether all detectable inputs or only suprathreshold inputs were counted. Thus, selective reinnervation of transplanted intercostal muscles is apparently based on positional differences in the rostro-caudal axis.

Many intercostal muscle fibers survive transplantation but others degenerate and are replaced by regeneration. To determine whether regenerating fibers are selectively reinnervated, T3 and T5 muscles were soaked for 0.5 h in the muscle toxin, bupivacaine (Marcaine; Carlson, Exp. Neurol. 52: 421, 1976) before implantation in the neck. This treatment killed all muscle fibers. New fibers regenerated, presumably from surviving satellite cells, and were successfully reinnervated by preganglionic axons. In this case, however, transplants from T3 and T5 did not differ significantly in the segmental pattern of their innervation. Thus, muscles that originated from different segments but regenerated in a common site were not distinguished by preganglionic axons, suggesting that while positional labels survive denervation and transplantation, their nature, amount, or accessibility can change during regeneration. (Supported by NSF and MDA.)

308.4 REPEATING PATTERN OF THREE FIBER TYPES IN A SINGLE-FIBER-THICK MUSCLE OF THE GARTER SNAKE. Jeff W. Lichtman and R.S. Wilkinson, Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, Missouri 63110.

Individual vertebrate muscles are unique with respect to the number, sizes, and types of motor units they contain. However, the mechanisms responsible for development of these stereotyped properties of muscle and nerve are unknown.

One approach to this problem is to study the pattern of fiber types and motor units in a relatively simple muscle at the level of individual cells. We have been studying an extremely simple muscle from garter snake in which local patterns of innervation may be quantitatively characterized. The snake transversus abdominus extends from each rib to the ventral midline, forming a single-fiber-thick continuous sheet along the ventrum of the animal; each segment contributes approximately 100 muscle fibers and its own innervating nerve. The muscle consists of three fiber types which can be distinguished by electrophysiological criteria (fast twitch, F; slow twitch, S; and tonic, T), fiber diameter ($d_F > d_S > d_T$), surface appearance, and color after gold chloride staining (F-blue, S-red, T-white). Tonic fibers have multiple endplates uniformly spaced along their length, while twitch fibers are innervated at solitary endplates which are 2-3 times larger than those of tonic fibers. In many regions of the muscle, fibers are arranged in the repeating pattern F-T-S-T-F-T, etc.

We have developed a method of stimulus-induced uptake of the enzyme horseradish peroxidase (HRP) which permits active endplates to be selectively labeled and, following silver intensification (1), to be visualized in the light microscope. Extracellular stimulation of a single motor axon at an endplate allows an entire motor unit to be labelled. The disposition of motor units of each type within the repeating pattern of muscle fibers is being studied.

Reference

Gallyas, F., T. Görcs and I. Merchenthaler (1982), J. Histochem. Cytochem. 30: 189-199.

This work was supported by a grant from the Muscular Dystrophy Association.

308.5 ACETYLCHOLINE RECEPTORS ARE PREFERENTIALLY INSERTED AND REMOVED AT CLUSTERS IN CULTURED RAT MYOTUBES. S. Bursztajn, S.A. Berman, J. McManaman and S.H. Appel, Neurology Department, Neuroscience Program, Baylor College of Medicine, Houston, Texas 77030

Noninnervated cultured rat myotubes contain clustered and diffuse acetylcholine receptors (AChRs). AChR clusters are essentially immobile, while diffuse AChRs are capable of lateral diffusion in the plane of membrane and may participate in cluster formation. In order to ascertain whether synthesis and insertion at preferential domains in the sarcolemma could account for the formation and maintenance of the cluster, we quantitated the spatial distribution of AChR addition and removal. Six-day-old rat myotubes were incubated with α-bungarotoxin (αBTX) conjugated to tetramethylrhodamine (TMR) to saturate all the AChRs initially present. The unbound fluorescent toxin was washed out and cultures were incubated in Dulbicco's Modified Eagle's medium (DMEM) at 37°C for 0h to 24h after which they were pulsed with ^{125}I αBTX (20nM). At each three hour time point cells were fixed and processed for autoradiography. This double label procedure allowed us to block surface AChRs; determine the distribution, and calculate the rate of appearance of newly inserted AChRs; and mark established, but not newly synthesized AChRs. Similarly, experiments were carried out which allowed us to localize and determine the rate of AChR removal from the clustered and diffuse region of the plasma membrane. In this case cells were labelled with ^{125}I-αBTX first, washed, incubated with DMEM for 0h to 24h, exposed to αBTX-TMR and processed for autoradiography. Diffuse and cluster AChR distributions as visualized by fluorescent αBTX and iodinated αBTX were quantitated with a computerized video image analysis system. The number of AChRs per um^2 of myotube surface membrane was always higher at the cluster than in the AChR diffuse region. The rate at which newly synthesized AChRs are inserted is 2 to 5 fold faster at the "old" AChR cluster than the diffuse AChR membrane region. During the first 2 to 4h, insertions of new AChRs occurred at the periphery of the "old" AChR cluster. By 18h the "old" cluster is completely filled with newly inserted AChRs, and the distribution of these AChRs within a cluster appears random. Measurements of AChR removal showed that AChRs are removed 2 to 5 fold faster from the cluster than from the diffuse region of the myotube surface. These results indicate that newly synthesized AChRs contribute to AChR cluster formation, and that the rate at which AChRs are inserted and removed occurs faster at the cluster than at the diffuse region of the plasma membrane.

Supported by NIH grant #NS 17876 and MDA to S. Bursztajn.

308.6 IN VITRO REGULATION OF MUSCARINIC ACETYLCHOLINE RECEPTORS BY AN ENDOGENOUS SOLUBLE FACTOR. T. L. Creazzo* and H. C. Hartzell* (SPON: E. Orona). Department of Anatomy, Emory University School of Medicine, Atlanta, Georgia 30322.

We have found an endogenous soluble factor which regulates muscarinic acetylcholine receptors (MAchR) in vitro in embryonic chick heart membranes. A 100,000 g soluble fraction was prepared from embryonic day-12 chick heart or brain and a 100,000 g crude membrane pellet was prepared from day 12 heart. Aliquots of membranes were incubated with varying concentrations of the labelled MAchR antagonist, ^{3}H-QNB, with or without the soluble fraction. The results, analyzed according to Scatchard, show that the factor produces a decrease in the number of MAchRs. In one particular experiment MAchR loss was as great as 90%. In addition, the affinity of the remaining receptors for ^{3}H-QNB is decreased 6 fold (control: Kd=8.5 pM; with factor: Kd=52 pM). We interpret these data in terms of hyperbolic noncompetitive inhibition by an endogenous soluble factor.

Partial characterization of the soluble factor indicates that the factor is heat and acid stable and less than 700 daltons. The factor is labile in under very stringent heat and acid conditions (i.e. 105°C in 6N HCl for 6 hours). The effect of the factor reaches an equilibrium in less than 6 hours at 25°C and the magnitude of the effect is linearly related to factor concentration. These findings suggest the factor is not a protease, nor an activator of a protease.

The concentration of factor is at least 5 fold greater in embryonic brain than in embryonic heart. No factor activity was detected in adult heart and only small amounts were present in adult brain. Our observations on the distribution of factor are in agreement with the work of other investigators who have shown that MAchR desensitization occurs in embryonic chick heart but not in adult heart. These data suggest that there is an endogenous soluble factor which participates in the regulation of MAchRs.

(Supported by NIH Grant HL21195.)

309.1 ADRENAL MEDULLARY HYPERPLASIA IN THE AGING LONG-EVANS RAT: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION. H.J. Wolfe[1]*, R.A. DeLellis[1], A.S. Tischler[1], and R.L. Perlman[2] (SPON: L.S. Adelman). [1]Dept. of Pathol., Tufts Univ. Sch. of Med., Boston, MA 02111; [2]Dept. of Physiol. and Biophys., Univ. of Illinois Coll. of Med, Chicago, IL 60680.

The Long-Evans rat has been reported to have a high frequency of age-related spontaneous hyperplasia or neoplasia of the adrenal medulla, thyroid C-cells and anterior pituitary. In order to characterize the adrenal medullary lesions, we have maintained a colony of male Long-Evans rats for more than three years. The animals were fed a standard laboratory diet and were sacrificed in groups corresponding to 9-12, 12-24, and 24-36 months of age. Serial sections of the adrenals were used for morphometric analysis of adrenal medullary volume, and additional samples were studied ultrastructurally. Catecholamine assays and cell culture studies were also conducted on some adrenals. Progressive diffuse and nodular adrenal medullary hyperplasia was observed to develop with increasing age. Compared to 9 to 12 month old animals, adrenals from the 12 to 36 month old groups showed two to three fold increases in medullary volume. In the older age groups, adrenal medullary cords appeared diffusely thickened. Nodules varying from 0.3 to 15 mm in diameter were found superimposed on these diffuse changes in 18% of 1 to 2 year-old animals, and 38% of 2 to 3 year-olds. These nodules were frequently bilateral and multicentric, and contained NE and DA with little or no E. In general, larger nodules tended to occur in older animals, and to show a marked degree of variation in cytological appearance by both light and electron microscopy. Small nodules had a more uniform appearance, and were composed of cells which resembled the Small Granule Containing (SGC) cells in normal murine adrenals. These cells contained electron-dense granules which were smaller than those of typical E- or NE-type cells, and sometimes formed interdigitating processes up to at least 3 cell diameters in length. These processes in vivo notably lacked the parallel arrays of microtubules which are observed in neuronal processes. Cells from adrenal medullary nodules showed spontaneous and NGF-induced neurite outgrowth in culture, but were heterogeneous in that regard. Our findings indicate that adrenal medullas of aging Long-Evans rats develop progressive diffuse and nodular hyperplasia of noradrenergic cells, some of which resemble SGC cells. Increasing magnitude of the hyperplastic changes is accompanied by increasing heterogeneity and by acquisition of some capacity for neurite outgrowth.

Supported by ACS Grant PDT-171; and NIH Grants CA27808, CA17389 and HL29025.

309.2 AGE-RELATED CHANGES OF DOPAMINERGIC RECEPTOR FUNCTIONS IN RAT BRAIN. E. Carboni*, M. Memo, V. Covelli, S. Govoni, M. Trabucchi and P.F. Spano. Institute of Pharmacology and Pharmacognosy, University of Cagliari and Milan, Italy.

It is clearly established that dopamine (DA) receptor system is affected by aging process. Particularly, we observed a significant decrease in dopamine stimulation of adenylate cyclase (AC) activity as well as in the number of dopamine receptors measured by ^{3}H-spiroperidol binding assay in striatum of aged rats as compared with young animals (Govoni et al., Brain Res. 138 (1977) 565). The present study was direct to investigate whether the changes in dopamine receptors represent a peculiar phenomena of aging.

Young (2 months), mature (12 months) and old (25 months) male Sprague-Dawley rats were used in our experiments. Dopamine receptor binding sites, labeled with ^{3}H-spiroperidol, and DA-stimulated AC activity were measured in striatum of all groups. We confermed the previously reported reduction in the number of ^{3}H-spiroperidol binding sites without changes in affinity in old rats as compared with young animals. Bmax values were 188±8 fmol/mg prot and 121±9 fmol/mg prot for young and old rats, respectively. Interestingly, striatal dopamine receptor density of mature rats was comparable with that of old rather the young rats (Bmax = 125±8 fmol/mg prot). The same pattern was observed measuring DA-stimulated AC activity. A significant decrease in cyclic AMP formation induced by DA was detect in striatum from either mature and old rats as compared with young animals.

Our results suggest that the decline in dopamine receptor function is not a peculiar phenomenon of aging but it appears even in mature rats. The levels of DA receptor binding sites and DA-stimulated AC activity do not change between 12 and 25 months old rats. It may be inferred that dopamine receptor function in rat CNS reaches stable levels during maturity and they are manteined in senescence.

309.3 MECHANISMS REGULATING DOPAMINE UPTAKE IN THE STRIATUM OF AGED RATS. C. Missale*, L. Castelletti*, S. Govoni, M. Trabucchi, P.F. Spano and I. Hanbauer. Dept. of Pharmacology, University of Brescia, Italy and NHLBI-NIH, Maryland 20205.

Changes in neurotransmitter content, metabolism and receptor activity in the brain have been invoked to explain age-dependent alterations of neural function. The dopaminergic neuronal activity is particularly impaired during aging. This rises the question on the regulation of the molecular events playing a cofactor role in the system. On these grounds the aim of the present investigation was to study the age-induced changes of the neuronal mechanisms regulating dopamine uptake. In particular, we investigated how aging affects the function of receptors labelled by cocaine. Two different populations of specific cocaine binding sistes have been detected in rat striatum according to their sensitivity to sodium ions. The Na^+-dependent cocaine binding site is preferentially located on dopaminergic terminals and is functionally related to the dopamine uptake system. The Na^+sensitive ^{3}H-Cocaine binding is decreased in senescent rats (Kd=1.7 ± 0.13 /uM; Bmax=10.1 ± 0.97 pmol/ mg protein and Kd=1.5 ± 0.15 /uM; Bmax 5.9 ± 0.44 pmol/mg protein for 3 and 25 month old rats, respectively); similarly the affinity of ^{3}H-Dopamine for its uptake system is also decreased in aged animals. A variation in the activity of this regulatory system may be a leading event in the age-dependent decrease of dopaminergic synaptic function. It has also been suggested the existence of an endogenous ligand for cocaine labelled receptors acting as a modulator of dopamine uptake. On this line, an age related defect of this endogenous modulator might be responsible for the observed alterations of dopamine uptake and cocaine binding.

309.4 AXONAL TRANSPORT OF CYTOSKELETAL PROTEINS IN AGING FISCHER RATS. I. G. McQuarrie and R. J. Lasek. Department of Developmental Genetics and Anatomy, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

The rate of transport for labeled cytoskeletal proteins has been reported to decrease in sensory axons of the sciatic nerve in aging Wistar rats (Komiya, Brain Res. 183:477-480, 1980). For the leading foot of labeled neurofilament triplet proteins, the rate at 7 months is 40% of the rate at one month, with a further 10% reduction occurring by 24 months. We have examined the transport of labeled cytoskeletal proteins in the sciatic nerve motor axons of Fischer rats, a strain that does not become obese or lethargic with increasing age. In these axons, the slowest of the five rate-components of anterograde axonal transport, termed SCa, principally contains the cytoskeletal proteins (neurofilament triplet, tubulin, actin) and is much more heavily labeled by radioactive amino acids than the next faster rate-component, termed SCb, which conveys a minor fraction of the labeled cytoskeletal proteins. Thus, the axonal transport of cytoskeletal proteins can be assessed simply by analyzing the amplitude and translocation rate of the SCa wave.

SCa was labeled by microinjecting ^{35}S-methionine or ^{3}H-lysine/proline into the motor column of the spinal cord; animals were sacrificed 20-40 days later. The rates of translocation for the peak of SCa (determined by dividing its distance from the spinal cord by the injection-sacrifice interval) at 3, 6, 12, 24 and 30 months were 1.3, 0.68, 0.43, 0.45, and 0.39 mm/day, respectively.

In young rats, 75% of SCa labeling is accounted for by the neurofilament triplet and tubulin, and the peaks of labeling for each of these polypeptides are co-transported (Hoffman & Lasek, J. Cell Biol. 66:351-366, 1975). Since these peaks are thought to represent the movement of cytoskeletal proteins in polymerized form (Tytell et al., Science 214:179-181, 1981), we wondered if the age-related slowing of SCa might be due to one of the cytoskeletal polymers moving more slowly than the other, thereby causing the whole complex to move more slowly through shear stresses. SDS-polyacrylamide gel electrophoresis was carried out to investigate this possibility. The peak of neurofilament triplet labeling was found to advance at 0.30 mm/day and 0.21-0.2 mm/day at 6 and 24 months, respectively. These rates were less than half of those for the peaks of labeled tubulin and actin. The rate for the neurofilament peak at 24 months was also measured rigorously using linear regression analysis for the 20-6 day interval, yielding a rate of 0.24 mm/day with a latent period (before the peak exited from the spinal cord) of 3 days.

Supported by NIH program project grant AG-00795.

309.5 FORMATION AND TURNOVER OF OLFACTORY BULB GRANULE CELLS IN THE YOUNG ADULT AND SENESCENT RAT. M. S. Kaplan* and J. W. Hinds. Dept. of Anatomy, Univ. of New Mexico, Albuquerque, NM 87131; Dept. of Anatomy, Boston Univ. School of Medicine, Boston, MA 02118.

Previous work (Kaplan and Hinds, '77 Science 197: 1092) has shown that new olfactory bulb granule cells can form in 3 month rats, but several questions remain: (1) Do most of the newly formed cells contribute to a permanent granule cell population or do most degenerate? (2)Is this new neuron formation restricted to young adult animals? (3) Is the rate of cell death different in senescent animals compared with young adults? To answer these questions two series of rats were injected with ^{3}H-thymidine (5 μCi/gm body weight, 20 Ci/mmole): (1) those injected at 3 and 24 months and surviving 1 to 18 months and 1 to 6 months, respectively; (2) those surviving 1 month after injection at six ages ranging from 3 to 30 months (median lifespan of rats 27 months). One micrometer plastic sections were cut 3000 μm from the rostral tip of the olfactory bulb. Slides were dipped in Kodak NTB-2 emulsion diluted 1:1 with distilled water, exposed at 4°C for 6 weeks in light-tight boxes, and developed in D-19. A cell was considered labeled if it had 4 grains over the nucleus, a value considerably above background.

Results to date bear on the first two of the questions posed above. The number of labeled granule cells per mm^2 granule cell layer surviving 1,2,3, and 6 months after injection in 3 month animals was found to decrease steadily over this interval, ranging from 20/mm^2 at 1 month to 6/mm^2 at 6 months; an apparent plateau or slow decrease then follows until 11 months survival (4/mm^2). No shift towards more lightly labeled cells at longer survival times was observed in histograms of the grain count distribution; thus the decrease in the labeling index is not a result of label dilution by repeated divisions. We conclude that about 3/4 of new granule cells formed at 3 months degenerate over a 6 month period, with about 1/4 surviving at least 11 months. Eighteen month survivals in this series are presently being analyzed, as are animals surviving 6 months after injection at 24 months. In a second series of animals new granule cells have been found labeled 1 month after injection, even in 24 and 30 month animals, but at much reduced numbers (about 3 labeled cells per mm^2 of granule cell layer compared with about 20 per mm^2 at 3 months). Intermediate ages are presently being studied to determine the time course of this decrease with age in formation of new granule cells.

(Supported by MBRS 5-506-RR08139-09 and NIA AG 00001)

309.6 ULTRASTRUCTURAL STUDIES OF AGING IN THE RAT MEDIAL NUCLEUS OF THE TRAPEZOID BODY. M. A. Casey and M. L. Feldman. Department of Anatomy, Boston University School of Medicine, Boston, Massachusetts 02118.

In an earlier light microscopic study we found that neuron loss occurs in the medial nucleus of the trapezoid body (MTB) throughout the adult lifespan of Sprague-Dawley rats (Casey, M. A. and Feldman, M. L., Neurobiol. Aging 3(3):187-195, 1982). We are now examining the MTB in rats 2 mo. to 33 mo. of age for age-related ultrastructural changes.

Consistant with our light microscopic findings, it was observed that age pigment (lipofuscin) accumulation in the neuronal cell bodies of old animals is relatively small. Electron microscopy does reveal, however, that in animals over 27 mo. of age the dendrites as well as the cell bodies of MTB cells may contain age pigment deposits. Of all age pigment deposits observed in the MTB, the largest ones are within glial cells and pericytes. In rats aged 24 mo. and older, basal laminae surrounding some blood vessels in the MTB contain large cavitations. Dendrites, large myelinated axons, and synaptic endings are all occasionally observed in varying stages of degeneration in the neuropil of the MTB in old animals.

Principal cells in the MTB synapse with two main types of axon terminals: (1) unusually large terminals (calyces of Held) forming multiple asymmetric synapses and containing large sperical synaptic vesicles, and (2) relatively small terminals forming symmetric synapses and containing densely packed small ovoid synaptic vesicles. Our preliminary quantitative studies indicate that the percentage of MTB cell somal surface covered by all terminals is reduced from about 58% to about 40% between 2 mo. and 27 mo. of age. The number of terminals per 100 um of somal perimeter decreases from about 27 to about 19 during the same period. Our data indicate that it is primarily the large terminals with multiple asymmetric synapses that are lost with age. Thus, in addition to neuron loss, there is an age-related partial deafferentation of principal cells in the rat MTB. Based upon the known source of the large terminals, it can be assumed that the synaptic loss affects the ascending auditory pathway originating in the cochlear nucleus. (Supported by NIA AG00001)

PAIN MODULATION: BEHAVIORAL ANALYSIS

310.1 PSYCHOPHYSICS OF THE TAIL-FLICK TEST: DIFFERENTIAL DRUG EFFECTS UPON LONG AND SHORT LATENCIES. Dennis D. Kelly. New York State Psychiatric Institute and Dept. of Psychiatry, Columbia University, New York, NY 10032

At a recent meeting of this Society, four of every five abstracts listed under the key words "pain" or "analgesia" employed the tail-flick test as the sole dependent measure of nociception. In most applications the intensity and location of the aversive stimulus were fixed, and a point-estimate of tail-flick threshold was reported. The following experiments suggest that these parameters cannot be set arbitrarily, for their values can determine both the degree and direction of the effect of an independent variable upon tail-flick latency.

In Exp I the intensity of a light focused 4 cm from the tip of the tail was varied systematically within sessions in 12 female rats. The resulting latency functions ranged from a median of 2.9 secs for tail withdrawal at the highest heat intensity to 10.3 secs at the lowest. Next the same rats were exposed in successive weeks to naloxone (10 mg/kg, s.c.), chlordiazepoxide (15 mg/kg, i.p.) and morphine (5 mg/kg, i.p.), each paired with a vehicle control session. Within each subject morphine's analgesic effectiveness was greatest upon rapid tail-flick latencies elicited by high intensity stimuli, and it fell off sharply as the light intensity used to elicit tail withdrawal was lowered. Naloxone significantly shortened long tail-withdrawal latencies to low intensity noxious stimuli, but had no effect upon rapid flicks to high intensity stimuli. Chlordiazepoxide extended tail flick responses only at an intermediate intensity and latency, not at either extreme.

In Exp II the intensity of light was fixed at the intermediate setting used in Exp I, and the location of the focus on the tail was varied systematically in 10 additional female rats. The sequence of exposure of five sites spaced 2, 4, 6, 8 and 12 cm from the tip was counterbalanced within sessions. Withdrawal latency to a constant light intensity was a monotone increasing function of distance on the tail, ranging from a median of 3.0 secs at a location 2 cm from the tip to 7.8 secs at 12 cm. Consistent with the results of Exp I, morphine (4 mg/kg) produced greater relative analgesia within the same subject when the tail flick was tested at more distal sites, whereas naloxone significantly shortened tail-flick latencies at only the two most proximal sites, associated with long latencies.

These data suggest that short and long tail-flick latencies are differentially sensitive to drugs. Hence the parameters that determine latency, such as the intensity and location of the stimulus, can also determine whether, and to what degree, an independent variable will induce analgesia, hyperalgesia, or neither.

(Supported by PHS Grant 1 R01 NS 18822)

310.2 THE INTENSITY OF MORPHINE ANALGESIA AND TOLERANCE DEVELOPMENT IN THE RAT DEPENDS UPON THE LOCUS OF TAIL STIMULATION. B.C. Yoburn*, D.D. Kelly, R. Morales* and C.E. Inturrisi* (SPON: M. Glusman). Dept. of Pharmacology, Cornell Univ. Medical College, New York, NY 10021; Dept. of Behavioral Physiology, New York State Psychiatric Institute, New York, NY 10032.

The rat tailflick test is widely used in the assessment of analgesic (antinociceptive) activity and tolerance development. We now report that different tail areas in the rostral-caudal axis are differentially sensitive to morphine. In three separate experiments the most distal portion of the tail compared to proximal locations was a more sensitive indicator of morphine analgesia and the development of tolerance. In the first experiment, 4 groups of rats were infused for 6 hours with saline or morphine (25, 35 or 45 ug/kg/min) through a jugular vein catheter. We assessed analgesia with the tailflick test at three 1-inch segments between 1 and 4 inches from the tip of the tail in a randomized fashion prior to and at 1, 2, 4 and 6 hrs during the infusion. The most distal location was significantly more sensitive to morphine than proximal locations as assessed by an earlier onset and greater intensity of analgesia and a slower rate of development of tolerance. Analysis of variance indicated significant dose, location, time and location x dose interaction effects (p's < .05). In a second experiment, rats were injected (sc) with saline, 2.5, 5.0 and 10.0 mg/kg morphine and analgesia was evaluated 1, 2 and 4 hr post-injection. As previously, we found a more rapid onset of analgesia and slower tolerance development when assessed at the distal location (significant dose, location, time and location x dose interaction effects, p's < .05). In a third experiment rats were infused via the jugular vein for 48 hr (45 ug/kg/min morphine) and significant location effects (p = .06) were observed. In none of these experiments did we find evidence of differences (p > .09) among locations during pretreatment testing. Furthermore, in a group of 86 rats tested before treatment, mean latencies were 7.1, 7.2, 7.1 sec for the 3 locations.

These results indicate that the tailflick test is liable to procedural constraints. Depending on the tail location and the dose we might conclude there is no drug effect, a marginal drug effect or a clear drug effect. It is obvious that these findings are of methodological importance especially in situations where the locus of stimulation is varied within an experiment, and in across experiment comparisons where tail location is not specified. Although the mechanism of this effect is not apparent, these data indicate that the extraordinarily useful tailflick test has properties that have not been recognized. Supported in part by DA-01457.

310.3 STRESS INDUCED ANALGESIA (SIA) IN MAN: MODULATION OF PAIN-RELATED BRAIN EVOKED POTENTIAL, A.C.N. Chen[1], B. Bromm[2] & R.-D. Treede[2]
[1]Psychiatry & Beh. Sci., University of Washington, Seattle, WA. 98195, USA; [2]Institute of Physiology, Hamburg University/UKE, 2000 Hamburg 20, West Germany.

Stress induced analgesia was evaluated in man using the submaximum effort tourniquet pain as a pathophysiological model. In human subjects, the brain evoked potential (BEP) has been shown to be a correlate of painful sensation (PS) of exogenous stimulation. This study aimed to evaluate how the BEP of phasic pain is modulated by concurrent subacute tonic ischemic pain through measurement of both BEP and PS.

Ischemic pain was produced in the right arm of the subject (n=10 subjects) with a blood pressure cuff inflated to 200 mmHg with a controlled exercise. Electrical noxious stimuli were delivered percutaneously to the tip of the middle finger in the left hand via needle electrode and PS was rated on a ten-point scale by the subjects. Pain-related BEP as well as prestimulus EEG were recorded from vertex. Recordings were digitized off-line on a PDP 11/34 computer. BEP was averaged from 40 stimulations in each record. Duration of ischemic pain lasted 15 min until reaching tolerance level.

Aching pain developed about 2 min after cuff inflation and then gradually increased to severe level within 5 min. A sharp intense pin-prick pain was invoked by the shock stimuli and a BEP could be consistently recorded in each trial. During concurrent contralateral ischemic pain, both the late P80-N130 and N130-P260 amplitudes were significantly attenuated while the early activity remained unchanged. Correspondently, subjects reported a decrease in pain rating to the shock stimuli. After releasing the pressure cuff, both BEP and PS returned nearly to the pre-ischemic levels.

These findings indicate that stressful subacute pain could modulate other noxious painful experience in man. Also, the subjective painfulness to the test stimuli could be reflected in the brain activity.

310.4 INTERMITTENT COLD WATER STRESS-INDUCED ANALGESIA (SIA): EFFECT OF AGE AND PREVIOUS CHRONIC STRESS. (SPON: J.A. Holloway). M.-N. Girardot* and F.A. Holloway. Department of Psychiatry & Behavioral Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, 73190 USA

In preliminary studies, the investigators found that intermittent cold water stress (ICWS) (2°C, exposure frequency 3/min, single duration 10 sec, total duration 3 min) induces a robust and consistent analgesia in rats, as measured ½ hour after stress by the tail-flick test. With chronic exposure to ICWS (daily, for 15 consecutive days), tolerance progressively develops. For instance, on a scale from 0 (no analgesia) to 1 (maximum possible analgesia) the analgesia indices (A.I.) drop from 0.6 on Day 1 to 0.1-0.2 on Days 4-15. Stress-tolerance is lost when chronically stressed rats are subsequently exposed to ICWS at two-week intervals for 3½ months. (Criteria for tolerance acquisition or loss: A.I. remains constant with 3 consecutive exposures to ICWS).

In the present study, possible long-term effects of chronic exposure to ICWS on SIA following subsequent acute or chronic stress were assessed. The experimental group was 5 rats previously chronically stressed (at 5 months of age) and further submitted to the loss of tolerance procedure by exposure at 2-week intervals for 3½ months. These animals were exposed to a second series of chronic ICWS at 15-16 months of age. The controls were 6 experimentally naive age-matched rats.

Two long-term effects of chronic exposure to ICWS on subsequent SIA were observed. (i) The animals with previous chronic stress experience were less analgesic following an acute exposure to ICWS than the age-matched naive animals (A.I.=0.6 and 0.9, respectively, $p<0.01$). (ii) ICWS-tolerance was acquired at a faster rate with the second than the first chronic exposure (4 vs. 6 days, respectively). Further, three effects related to age were observed: (i) Acute exposure to ICWS induced significantly more analgesia in the 15-16 month old than in the 5 month old animals (A.I.=0.9 and 0.6, respectively). (ii) ICWS-tolerance was acquired only after 17 days of chronic exposure in the 15-16 month old rats not previously stressed (vs. 6 days in the 5 month olds). (iii) At maximum tolerance, A.I. was identical in both the 15-16 month old groups and significantly higher (A.I.=0.3-0.4) as compared to the younger rats submitted to chronic ICWS (A.I.=0.1-0.2). The opiate antagonist naltrexone (10 mg/kg) did not significantly block this age-related residual analgesia, suggesting that it is not mediated by endogenous opioids ($p>0.1$).

These various results demonstrate that when SIA is measured, (i) chronic stress reduces the reactivity to acute stress and accelerates the rate of acquisition of stress-tolerance, and (ii) aging has the opposite effect. Aging further prevents development of complete stress-tolerance.

310.5 THYROTROPIN RELEASING HORMONE: POTENTIATION OF DIFFERENT FORMS OF FOOT-SHOCK ANALGESIA. P. D. Butler and R. J. Bodnar. Dept. of Psychology, Queens College, C.U.N.Y., Flushing, NY 11367.

Thyrotropin releasing hormone (TRH) appears to interact with both opioid and non-opioid systems in mediating hypothermic, hypoactive, cataleptic, respiratory, and analgesic effects. While TRH neither antagonizes opioid analgesia nor alters pain thresholds itself, it blocks neurotensin analgesia. Either opioid or non-opioid pain inhibitory systems can be activated by selectively altering the parameters of shock. For instance, while analgesia induced by twenty inescapable tail-shocks delivered on a variable interval 1 min (VI-1) schedule is not reversed by naltrexone, a greater number of shocks (60 or 80) does elicit naltrexone reversible analgesia (Science, 213, 1409, 1981). The first experiment examined whether TRH would alter the elevations in tail-flick latencies induced by 20 and 80 foot-shocks. Three groups of eight rats received icv injections of 0, 10, or 50 ug/10 ul of TRH respectively immediately before exposure to 20 foot-shocks delivered on a VI-1 schedule. Twenty shocks paired with vehicle significantly elevated tail-flick latencies over baseline for up to 5 min post-shock. The TRH doses of 10 and 50 ug significantly lengthened the duration of analgesia induced by 20 shocks for up to 8 min and up to 15 min post-shock respectively. Moreover, the magnitude of analgesia induced by 20 shocks was significantly potentiated in a dose-dependent manner by TRH. Another three groups underwent an identical paradigm, except that 80 shocks were delivered and injections were administered immediately before the last 20 shocks. As in the 20 shock condition, TRH significantly and dose-dependently potentiated the duration and magnitude of tail-flick latency elevations induced by 80 shocks paired with vehicle. Experiment 2 extended these findings to a visceral pain test, the writhing test. While rats exposed to 80 shocks and vehicle failed to display changes in the number and intensity of writhes from baseline, the pairing of 80 shocks with TRH (50 ug) induced analgesia as measured by significant decreases in the number (vehicle/shock: 4.9; TRH/shock: 2.5) and intensity of writhes. These data suggest that TRH is involved in the modulation of both opioid and non-opioid forms of analgesia. Supported by NIH GRSG 5-S05-RR-07064.

310.6 EVIDENCE OF GLYCINERGIC MEDIATION OF VAGINAL STIMULATION-PRODUCED ANALGESIA IN RATS. C. Beyer*, L. Roberts*, and B.R. Komisaruk. Univ. Auto. Metro-Ixtapalapa, Mexico City, Mexico, and Institute of Animal Behavior, Rutgers Univ., Newark, New Jersey 07102

Vaginal-cervical mechanostimulation (VS) has been shown to suppress vocalization and withdrawal responses to noxious stimulation, an effect significantly reduced, but not abolished, by procedures which block monoaminergic neurotransmission (e.g. spinal transection, dorsolateral funiculus lesions, systemic or perispinal administration of phentolamine or methysergide) or endogenous opiate action (systemic or perispinal naloxone administration) (review: Komisaruk, B.R., in Brain Stem Control of Spinal Mechanisms, B. Sjolund and A. Bjorklund, (Eds.), p.493, 1982, Elsevier, N.Y.). In order to determine whether the inhibitory neurotransmitter, glycine, contributes to VS-produced analgesia, strychnine, a specific glycine receptor antagonist, was administered perispinally via an indwelling catheter. Strychnine sulfate alone induced dose-dependent sensory and motor effects. The 5µg dose (in 5µl) was subconvulsive and produced recurrent episodes of grooming, scratching and biting of the skin, starting 1 min post injection and persisting approx. 10 min. For approx. 30 min, vocalization hyperresponsiveness occurred to gentle stimulation of the fur or skin (using a jet of air or smallest diameter von Frey fibers). Higher doses of strychnine (25µg, 100µg) increased the intensity and duration of these effects, producing motor seizures (kicking, jumping, rolling). Prior to strychnine administration, VS elevated current thresholds for vocalization to tail shock and blocked vocalization elicited by stimulation of a skin area previously sensitized by a 0.5ml intradermal injection of 20% yeast solution. After strychnine administration at each of the above dosage levels, these effects of VS were markedly attenuated. However, VS was effective in suppressing the motor seizures produced by strychnine. These data suggest that glycine mediates the analgesic effect of VS. Supported by NSF Grant BNS 8217702 (BRK).

310.7 CHEMICALLY INDUCED ITCH, PAIN AND HYPERALGESIA BY INTRAEPIDERMAL INJECTION. L. Becerra-Cabal,* R.H. LaMotte, J. Ngeow* and G.J. Putterman* (SPON: L. Marks). Depts. of Anesthesiology and Ophthalmology, Yale Univ. Sch. of Med., New Haven, CT 06510

We have used chemical substances selectively to produce itch, pain and hyperalgesia using a technique (intraepidermal injection) which can also be applied to neurophysiological studies of cutaneous nociceptors.

Psychophysical scaling techniques were used (as approved by the University Human Investigation Committee), to obtain continuous judgments in 10 humans of the magnitude of itch and pain sensations after a single intraepidermal injection in the forearm of 10µl of different concentrations of histamine, bradykinin, kallikrein, or capsaicin. Brief heat stimuli of 30-48°C were also periodically delivered to the injected skin.

Histamine produced itch, not pain, which monotonically increased in magnitude and duration with increases in the amount of histamine (.001 to 1 µg but not beyond). Histamine had little effect on heat pain. Bradykinin (90µg/ml) and kallikrein (20 IU/ml) produced itch, often with stinging pain, and transient decreases in heat-pain threshold and increases in suprathreshold heat pain.

Capsaicin produced burning pain, and not itch, and had a median absolute threshold of 0.1µg. The magnitude of pain increased monotonically with the amount of capsaicin (1 to 100µg). The mean absolute threshold for heat pain decreased from 41.6°C (normal) to 33.3°C. Suprathreshold heat pain was greater than normal as evidenced by a decreased latency of pain, large increases in magnitude ratings and durations of pain, and lowered thresholds for detecting - and rating as painful - small increments in temperature (0.1-0.3°C) delivered on an adapting temperature of 36°C. An abnormal sensation of pain or unpleasantness was produced by rubbing the skin, slight movement of single hairs or von Frey stimulation - particularly after 10-100 µg of capsaicin. The persistence of altered pain states after capsaicin was dose related, largely disappearing within 30 min after 10µg or less but not until 2-3 hrs after 100µg.

We conclude that chemogenic itch and pain can be quantitatively evoked and measured. Further, the magnitude and time course of chemically-induced pain and hyperalgesia can be measured via application of heat stimuli to the skin. Thus these techniques should be applicable in combined psychophysical studies of sensation and electrophysiological studies of cutaneous nociceptors.

Supported in part by USPHS Grants NS 14624 and EY 00237

COMPARATIVE NEUROANATOMY I

311.1 MIDBRAIN TECTAL AFFERENTS IN THE BLIND CAVE FISH, *ASTYANAX HUBBSI*. S. E. Fish, T. J. Voneida and B. K. Weston*. Northeastern Ohio College of Medicine. Neurobiology Program, Rootstown, Ohio 44272.

The "optic" tectum of the blind cave fish *(Astyanax hubbsi)* has a small but nonfunctional retinal input. One might expect that the loss of this primary afferent would result in changes in the tectum, but with the exception of some shrinkage, the general organization and efferent connections are similar to those reported in normally sighted teleosts. With these facts in mind, we have plotted the afferent connections of the cave fish's optic tectum using the horseradish peroxidase (HRP) method.

Following injections of HRP into the optic tectum, cells filled with the reaction product were located ipsilaterally in the dorsal posterior, central posterior and ventral medial nuclei of the thalamus (terminology from Northcutt). Tectal afferents from the pretectum were also ipsilateral and arose from neurons in the pretectal nucleus and central pretectal nucleus. A few labeled cells were also found in the superficial pretectal nucleus. In the midbrain the most consistent finding was a bilateral projection from the lateral nucleus of the torus semicircularis where filled cells were found in two laminae. Ipsilateral afferents from the nucleus isthmi, rostral tegmental nucleus and other unidentified tegmental nuclei were also found. Only occasional HRP filled cells were located in the torus longitudinalis and deep layers of the contralateral tectum. Brainstem projections were found to arise bilaterally from the superficial reticular formation and contralaterally from the nucleus of the descending trigeminal tract.

In general the pattern of tectal afferents described above is similar to that in other ray-finned (actinopterygian) fishes. One finding, the projection from the nucleus of the descending trigeminal tract, is of particular interest, since we have previously demonstrated a somatic representation in the tectum with electrophysiological methods. This projection in teleosts has not previously been identified with modern methods and may not be present in *Astyanax mexicanus*, the cave fish's sighted ancestor (preliminary result). If the cavefish is unusual among teleosts in having trigeminal input to the optic tectum then this pathway may represent an evolutionary adaptation to the cave environment.

This work was supported by NIH Grant NS18369.

311.2 ORIGINS OF DESCENDING SPINAL PROJECTIONS IN LUNGFISHES. Mark C. Ronan and R. Glenn Northcutt. Neurosciences Program and Div. of Biol. Sci., University of Michigan, Ann Arbor, MI. 48109.

Cells of origin of the descending spinal projections in African lungfishes, Protopterus spp., were identified by retrograde transport of HRP introduced into the rostral spinal cord. In three juveniles (25-30cm body length), gelfoam pledgets, saturated with 40% HRP (Sigma VI) in distilled water, were placed in approximate spinal hemisections near the fourth cervical segment. The spinal cords of two other Protopterus juveniles were unilaterally inoculated just caudal to the obex with thickened HRP paste on the tip of a #-000 insect pin. After 8, 12, or 15 days survival at 24-26°C, the animals were perfused and 35-40 micron thick transverse sections of the brains and rostral spinal cords were processed according to modified TMB (Mesulam, '78) or Hanker-Yates (Hanker et al., '77) protocols. Subsequently, two juvenile specimens of the South American lungfish, Lepidosiren paradoxa, received unilateral HRP innoculations between the second and third spinal segments. Animals survived 8 days at 25-27°C; brains and rostral spinal cords were sectioned horizontally at 40 microns and processed with modified TMB or Hanker-Yates methods.

The origins of descending spinal projections were similar in all animals examined. In the mesencephalon, labeled cells were present in the nucleus of the MLF, the pretectum, and the superficial layer of the optic tectum. HRP-positive tectal cells were located mostly contralateral to the labeled side of the spinal cord. A few labeled cells were observed in the mesencephalic trigeminal nucleus.

Labeled cells were also present in the superior, middle, and inferior divisions of the medullary medial reticular nuclei, the inferior raphe, the magnocellular vestibular nucleus, the nucleus of the solitary tract, and the nucleus of the descending trigeminal tract. Medullary neurons were labeled bilaterally, but were more numerous on the ipsilateral side. Ipsilateral labeling of reticular nuclei was most pronounced in the caudal and middle medulla and diminished rostrally at the level of the superior reticular nucleus. Contralaterally, HRP-positive cells were located along the ventral and lateral borders of the inferior reticular nucleus. Rostral to the level of HRP implants, labeled cells were present in the spinal cord; most were found in the intermediolateral spinal gray on the contralateral side.

In conclusion, the origins of spinal projections descending from the brain stems of lungfishes resemble many of those reported for other jawed vertebrates. The reticulospinal system, including an interstitiospinal projection, is likely the largest source of descending input to the spinal cord.

(Supported by NIH Grant NS 11006 to RGN).

311.3 USE OF COBALT-LYSINE FOR TRACING THE CONNECTIONS OF ELECTRORECEPTIVE NUCLEI IN AN ELASMOBRANCH. J. Schweitzer and D. A. Lowe.* Neurobiol. Unit, Scripps Inst. of Oceanog., Univ. of Calif., San Diego, CA 92093.

Horseradish peroxidase is poorly transported in elasmobranchs, hence experiments with sharks and rays require survival times of up to 3 weeks. Likewise radioactive amino acid tracers have a disadvantage in requiring processing times of up to 2 months. These considerations led us to explore the usefulness of Co^{2+}-lysine as a neuroanatomical tracer in the elasmobranch CNS. The method has proved valuable in determining central connections of electrosensory nuclei of the thornback ray, Platyrhinoidis triseriata. We are able to visualise a large number of labelled axons and their collaterals, the morphology of the cells from which they project, as well as details of terminal branching within restricted target areas, after survival times of 2-48 hours. A similar method has been used recently to reveal tectal pathways in Rana (Lazar, G. et al, JCN, 215:108, 1983).

Glass recording electrodes filled with Co^{2+}-lysine were stereotaxically placed into the lateral mesencephalic electroreceptive nucleus, LMN, while applying a negative backing current. Electrode position was confirmed by recording multiunit electroreceptive activity in response to uniform electric fields, and the cobalt complex then iontophoretically injected into the recording area. Subsequently, Co^{2+} was precipitated by transcardial perfusion of $(NH_4)_2S$ in elasmobranch Ringers, the animal fixed by perfusion with alcohol-formalin-acetic acid and the brain embedded in paraffin. Twenty-micron sections mounted on glass slides were intensified using standard developers (Tyrer, N.M. and Bell, E.M., Brain Res., 73:151, 1974; Gallyas, F., Stain Technol., 54:173, 1979) and then counterstained with neutral red. Uninjected rays, similarly treated, controlled for endogenous metals and showed no background activity.

LMN injections result in the labelling of axons and terminal boutons in the posterior lateral thalamic nucleus, PLT, a diencephalic electroreceptive region (Schweitzer, J., Neurosci. Abstr., 8:1026, 1982), demonstrating a direct LMN-PLT projection. LMN injections also result in labelling of many commissural fibers that course from the injection site to terminate in the contralateral LMN. Other fibers in the commissure may represent fibers of passage from the ipsilateral dorsal octavolateralis nucleus (DON) to the central tectal zone, described in Raja on the basis of degeneration and proline studies (Boord, R.L. and Northcutt, R.G. JCN, 207:274, 1982). Retrogradely labelled ascending lemniscal fibers can also be traced caudally to the contra- and ipsilateral DON.

(Supported by NIH and NSF grants to T.H. Bullock, an MRC grant to I.J. Russell, and a Royal Society travel grant to D.A. Lowe).

311.4 INNERVATION OF THE MUSCLE OF OCULAR ACCOMMODATION IN A FOVEATE TELEOST. J. C. Wathey. Neurobiology Unit, Scripps Instit. of Oceanography and Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093.

The predatory serranid bass Paralabrax clathratus has a highly specialized visual system and a large range of ocular accommodation (Schwassmann, H. O., In: M. Ali (ed.), Vision in Fishes pp. 279-288, Plenum, 1975). As part of an ongoing study of the central control of accommodation in this species, I have examined the innervation of the muscle which effects this behavior, the retractor lentis.

The short ciliary nerve was traced to its origin, both in gross dissections and in Klüver-Barrera stained serial sections taken from fixed and decalcified heads. I confirm the earlier anatomical descriptions of the ciliary, trigeminal sympathetic and profundus ganglia as seen in other teleosts (Young, J. Z., Proc. Roy. Soc. B. 107: 464-485, 1931; Meader, R. G., J. Morph. 59: 163-172, 1936). In three animals horseradish peroxidase (Sigma VI) was applied to the cut short ciliary nerve several millimeters proximal to the retractor lentis; at this location the nerve also contains some fibers that innervate the cornea and iris. Labeled neurons were observed in the ciliary, trigeminal sympathetic and profundus ganglia after survival times of 9 or 10 days. No cells were labeled in the brain. To determine the destination of the sympathetic fibers, retractor lentis and iris were examined in three animals using glyoxylic acid induced fluorescence to demonstrate noradrenergic fibers and terminals. The iris receives a substantial noradrenergic innervation, while the retractor lentis is devoid of such fibers.

The present findings indicate that a dual innervation, such as that known to exist in the primate ciliary muscle, does not occur in the teleost retractor lentis.

(This work was supported by an NSF graduate fellowship to the author and by NSF and NIH grants to Dr. T. H. Bullock.)

311.5 RUBROSPINAL PATHWAY IN A COLUBRID SNAKE. W. L. R. Cruce, L. Larson-Prior and D. B. Newman. Neurobiology Program, NEOUCOM Rootstown, Ohio 44272 (WLRC, LL-P) and Anatomy Dept., USUHS, Bethesda, Maryland 20814 (DBN).

Four water snakes (Nerodia, formerly Natrix: 3 N. fasciata fasciata and 1 N. taxispilota) received unilateral injections of 0.2-0.75µl of 25% horseradish peroxidase (HRP, Worthington) in the spinal cord at cervical and thoracic levels (18th to 40th segments). After 5 to 6 days the brains were treated with the TMB or DAB HRP methods. In the rostral mesencephalon (level of interpedunclular n., rostral to oculomotor n., caudal to posterior commissure) three tegmental nuclei (n.) were filled with HRP. The interstitial n. (IN) consisted of large (23 x 32µ multipolar neurons containing coarse Nissl granules. The cells were scattered lateral to the ventricle and to the medial longitudinal fasiculus (MLF). The n. of the MLF (NMLF) consisted of medium (15 x 30µ), spindle-shaped neurons with little Nissl substance. These were tightly packed on top of and medial to the MLF. The IN and NMLF were labelled ipsilaterally; contralateral label was found in a tight cluster of cells ventral and lateral to the above two nuclei. This nucleus was made up of small (12 x 20µ) multipolar neurons with large Nissle granules. Its topographical location and its contralateral projection to the spinal cord leave little doubt that this is the cell group called the red n. (RN) in other reptiles. The size of this RN is smaller than that seen in many limbed reptiles and particularly striking is the small size of its neurons (limbed reptiles generally have a RN made up of small, medium, and large neurons). Ten Donkelaar (Prog. Brain Res. 57:25-67, 1982) has asserted that the rubrospinal pathway does not exist in animals without limbs, especially snakes. He studied Python, a boid snake, which is "primitive", that is sharing many characters (e.g. reduced visual system, presence of pelvic girdle) with the ancestral burrowing lizards from which snakes evolved. Nerodia is a colubrid snake which is more "advanced". Since colubrids have no pelvic gridle, they are even further removed from the limbed condition and, one would suppose, even less likely to have a rubrospinal pathway. However any given snake may have a complex assortment of primitive and advanced characters and the colubrids, in particular, have secondarily evolved a well-developed visual system. If the rubrospinal pathway proves to be widespread in colubrids but not in boids, it could be an example of homoplasy. In this case, it would not be surprising to find differences in the pathway, e.g. that it was no longer involved in limb function. This raises the question of whether the rubrospinal pathway in mammals has any function not involved in limb control.

311.6 A CAUDAL STRIATAL DIVISION IN RANID FROGS. T.J. Neary. Anatomy Dept., Creighton Univ., Omaha, NE 68178

Traditionally, two major divisions of the anuran striatal complex have been recognized -- the dorsal and ventral striatum. Together, they form the greater part of the complex. Recent evidence, however, supports recognition of a caudal division which includes parts of both traditional divisions. This caudal division is characterized connectionally by its strong projections to the obex region (Neary, '82) and histochemically by a moderate, perilaminar AChE-positive zone. Following injections of the caudal striatum in ranid frogs with peroxidase-conjugated wheat germ agglutinin, the following connections were found: 1) Inputs. Most primarily ipsilateral. Ventromedial portion of the medial pallium, anterior and central thalamic nuclei, anterior division of the lateral thalamic nucleus, suprachiasmatic nucleus (two populations), ventral and lateral hypothalamus, posterior tuberculum, pretoral grey, anteroventral tegmental nucleus, superficial isthmal reticular nucleus, cells medial to the oculomotor nucleus, secondary visceral nucleus, parabrachial nucleus, and cells ventromedial to the solitary tract (these were primarily contralateral). A few cells were also found in the contralateral caudal striatum. 2) Outputs. Fibers could be followed through the lateral forebrain bundle. All projections were ipsilateral with the exception of a strong projection to the contralateral anterior entopeduncular nucleus and a weak one to the contralateral caudal striatum. A moderate projection could be followed to the posterior thalamic nucleus and the posterodorsal division of the lateral nucleus and a light projection to the pretoral grey was evident. Two very dense terminal areas were seen in the mesencephalic tegmentum, one ventral to the anterodorsal nucleus and the other lateral to the anteroventral nucleus. These areas show a low AChE activity. In the isthmal region, fibers coursed through the lateral and periventricular white matter near the parabrachial nucleus and the lateral reticular grey and some appeared to terminate in these areas. Labelled fibers could be followed through the lateral white matter of the medulla to the obex region where they entered the grey matter ventromedial to the solitary tract. A few fibers could also be followed into the spinal cord, coursing through the dorsomedial part of the lateral funiculus as far as S3. Most appeared to enter the intermediate grey. These results confirm and extend the recent findings of Wilczynski and Northcutt ('83).

Supported by NSF Grant BNS-7924699.

311.7 TOPOGRAPHIC DISTRIBUTION OF AFFERENTS TO THE CEREBELLUM IN A FROG (Rana esculenta) AS REVEALED BY RETROGRADE TRANSPORT OF WHEAT GERM AGGLUTININ CONJUGATED WITH HORSERADISH PEROXIDASE.
B.G. Grover* (SPON: T.E. Frumkes). Dept. of Physiol., Freie Univ. Berlin, 1000 Berlin 33, West Germany.

Pressure injections of 3% wheat germ agglutinin conjugated with horseradish peroxidase (Sigma) were made via micropipettes into various parts of the cerebellum in ten frogs. Survival times of 3 to 10 days were allowed. Alternate series of cryostat sections were processed on the slide with diamino-benzidine and benzidine dihydrochloride (BDHC). Cases were obtained in which the dense core of the injection site (re BDHC) was restricted to anterior, posterior and auricular parts of the cerebellum with the following results.

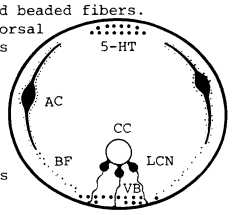
Somatosensory: Ipsilateral labelling in the principal sensory and spinal trigeminal nuclei and bilateral labelling of cells in the ventral horn at all levels of the spinal cord down through the lumbar enlargement was found following injections restricted to or including the anterior medial cerebellum. Vestibular: Bilateral labelling in the medial and descending vestibular nuclei was found following injections restricted to or including either the posterior medial cerebellum or auricle. Reticular: 3 groups of cells in the caudal rhombencephalon were labelled bilaterally after larger injections involving the entire medial cerebellum. In the rostral rhombencephalon, cells at the level of the superior olive were heavily labelled following injections of the auricle. Periventricular cells in the rostral mesencephalic tegmentum were labelled in all cases. Molecular layer afferents: cells in the contralateral inferior olive and in the ventral raphe at this level were the only cells labelled after injections restricted to the molecular layer. There is some suggestion of topographic organization in the projection of these cells onto the cerebellum.

The topographic distribution of afferents to the frog cerebellum is in many points similar to that found in birds and mammals. The distribution of afferents from the rostral rhombencephalon in particular, raises the question as to whether the auricular lobe of cold-blooded vertebrates represents a 'neo-' or ponto-cerebellum as it does in birds.
Supported by grant GR 276/19 from the Deutsche Forschungsgemeinschaft.

311.8 TYROSINE HYDROXYLASE AND SEROTONIN DISTRIBUTION IN THE FROG FILUM TERMINALE.
M. Chesler, P. Thompson* and C. Nicholson, Dept. Physiol., New York Univ. Med. Ctr., 550 First Ave., New York, NY 10016

The filum terminale of the frog is the most caudal region of the spinal cord and contains predominantly glial elements. The presence of numerous axon profiles in transverse section (Ariens Kappers, Huber and Crosby, The Comparative Anat. of the NS of Vertebrates Incl. Man, 1936) suggests that the anuran filum terminale may serve a functional role. We have studied the distribution of tyrosine hydroxylase (TH) and 5-HT immunoreactivity in the filum and more rostral spinal cord of Rana catesbeiana. Immunocytochemical staining has revealed a characteristic distribution of aminergic perikarya and fibers.

Perikarya of the CSF or liquor contacting neurons (LCN) and their processes stained intensely for TH. These 6-8 µm flask-shaped, bipolar cells (Vigh and Vigh-Teichmann, Int. Rev. Cytol. 35: 189, 1973) were located exclusively in the ventral ependymal and subependymal layers and were found at every spinal level as well as the medulla. A single dendritic process contacted the ventral border of the central canal (CC) while the axon extended to the ventral-medial surface of the cord to end as a sub-pial knob. In the filum these knobs formed a dense strip of TH reactivity below the pia. Additional TH reactivity was confined almost exclusively to a ventral band (VB) of fibers located immediately above the sub-pial knobs. Occasional beaded fibers (BF) arose bilaterally from this band and ran a dorso-lateral course to envelope a group of expansive arcuate cells (AC) in the dorso and ventro-lateral aspect of the distal filum. The arcuate cells did not stain for TH but their somata (15-20 µm) and processes were outlined distinctly by beaded TH positive fibers. It seems likely that LCN collaterals are the source of the VB and beaded fibers. 5-HT reactivity was found mainly in a dorsal cluster of fibers. Isolated 5-HT fibers were also seen throughout the filum. These findings suggest the presence of local neuronal circuitry and a possible neuroendocrine role for the anuran filum. TH antibody was kindly provided by Dr. T.H. Joh, Cornell Univ. Medical Coll. Supported by USPHS Grants NS-13742 and 5-T32-GM-07308.



311.9 EVIDENCE FOR A GABA-CONTAINING STRIATO-TEGMENTAL PATH IN PIGEONS.
K. Hall*, S. E. Brauth and C. A. Kitt, Department of Psychology, University of Maryland, College Park, Maryland 20742.

Well developed striato-tegmental pathways have been described in all amniote species studied to date. In amniotes these pathways originate from neurons contained within small-celled portions of the corpus striatum (paleostriatal complex of reptiles and birds) and terminate in relation to catecholamine-containing cell groups of the midbrain tegmentum. The striato-tegmental pathways of mammals, pigeons, caiman and turtles have been shown to contain substance P fibers derived from substance P-positive neurons in small-celled portions of the striatal complex. In mammalian forms the striato-tegmental pathways have also been shown to contain gamma aminobutyric acid (GABA). The existence of GABAergic projections within the striato-tegmental systems of sauropsids has never been demonstrated.

In this study, GABAergic connections in the striato-tegmental system of the pigeon were investigated by means of the retrograde transport of tritiated GABA (Streit et al, Science, 1979). This method utilizes light microscopic autoradiography to detect the presence of transported label. Injections of ^{3}H-GABA were placed in the midbrain tegmentum of pigeons, centered within the nucleus tegmenti pedunculopontinus (TP). TP of pigeons has been shown to be comparable to the mammalian nigral complex on the basis of cytoarchitectonic, histochemical and hodological criteria.

The results show that label was transported from the injection site to many paleostriatal fields. Heavy silver grain label was observed over cells and fibers of the ventral paleostriatum (VP), a region through which the medial forebrain bundle passes, as well as over neurons contained in the nucleus accumbens (Ac) and the ventral rim of the lobus parolfactorius (LPO). Light label was seen in the dorsal portions of the LPO. The paleostriatum augmentatum (PA), paleostriatum primitivum (PP) and nucleus intrapeduncularis (INP) contained grain label which was slightly above tissue background. Previous studies (Kitt and Brauth, 1981) have shown that all of these fields project upon the midbrain tegmentum in the pigeon, although only the LPO and PA contain substance P-positive neuron cell bodies (Reiner et al, Neurosci., 1983).

The present results therefore provide evidence that GABAergic neurons contribute to the striato-tegmental projection system of the pigeon. As in mammals, there is overlap between the striatal fields containing substance P-positive neurons and those contributing to the GABA projections. These data therefore provide additional evidence for the theory that the striato-tegmental system arose at an early point in amniote evolution and has been retained by extant species in this vertebrate group.
Supported by Grant No. NS 13018 to Dr. Brauth.

311.10 RELATIONSHIP OF AREA POSTREMA TO THREE PUTATIVE MEASURES OF "MOTION SICKNESS" IN THE RAT.
R. Sutton*, R. Fox, and N. Daunton. San Jose State Univ. and NASA Ames Res. Center, Moffett Field, CA, 94035.

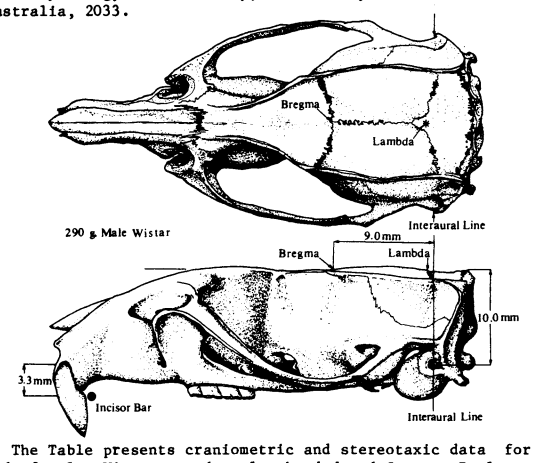
Although the rat has an incomplete emetic reflex, several species-specific responses to motion have been proposed as measures of "motion sickness" in rats. The purpose of this study was to determine the dependence of these responses on one of several neural structures known to be essential to motion-induced vomiting in species with a complete emetic reflex.

The Area Postrema (AP) has been shown to play an important role in the production of motion sickness in vomiting species (Brizzee, et al., 1980; Wang and Chinn, 1954). In this study we compared the effects of thermo-cautery ablations of the AP on three different responses supposedly reflecting motion sickness in the rat: Conditioned taste aversion [CTA] (Braun and McIntosh, 1973); drinking suppression (Haroutunian, et al., 1976); and fecal boli (Ossenkopp, 1983). Efficacy of the ablations was determined by subjecting ablated, sham-operated, and unoperated control animals to a CTA test which is known to require a functional AP. Animals with AP ablations failed to form CTA when 0.15 M LiCl was paired with a 10% sucrose solution, while sham-operated control subjects conditioned as well as the unoperated control subjects. The extent of the ablations was evaluated histologically at the end of the experiment.

To determine the effects of the ablations on the measures of motion sickness, all animals were subjected to rotation for 30 min or 90 min on a platform displaced 20 deg from earth horizontal. Results indicate that ablation of AP in the rat has no effect on the formation of CTA to a 4% solution of cider paired with motion, on the suppression of drinking immediately after exposure to motion, or on the frequency of fecal boli during exposure to motion.

This failure of AP ablations to eliminate the effects of motion on any of these responses discourages their use as equivalents of motion-induced vomiting. The appropriateness of other suggested measures, e.g., pica (Mitchell, et al., 1976), remains untested, but the dependence of such measures on stimulation more severe than commonly used in motion sickness research and the absence of a demonstration of their dependence on neural structures essential to motion sickness in vomiting species, suggest caution in the use of such responses. Further, until more is known about the neural structures underlying these putative measures, the rat will remain a questionable subject in which to study motion sickness.

311.11 CRANIOMETRIC COMPARISONS BETWEEN RATS OF DIFFERENT SEX AND STRAIN. George Paxinos* and Charles Watson*, (SPON: D. Rapaport) Schools of Psychology and Anatomy, University of New South Wales, Australia, 2033.



The Table presents craniometric and stereotaxic data for male and female Wistar, and male hooded and Sprague Dawley rats of 280-305 g weight. With the head in the flat skull position, 10 rats from each group were subjected to stereotaxic insertion of needles according to the procedure of the atlas of Paxinos and Watson (The Rat Brain in Stereotaxic Coordinates. Sydney: Academic Press, 1982). Bregma and lambda were redefined as the midpoints of the curves of best fit along the corresponding sutures. In all groups, bregma (B) was directly dorsal to the anterior commissure (ac) and thus more stable for localization of anterior structures, whereas the interaural midpoint (I) was more stable for posterior structures. In the Table, anteroposterior (AP) and dorsoventral (DV) distances are in mm (g7 = genu of facial nerve; L = lambda, all SDs < 0.5). The variability between different strains and sexes is well within the range obtained within any one group. Supported by Australian ARGC D18015147R and NH & MRC 820513.

	AP I-B	AP I-L	DV I-B	AP B-ac	AP I-g7	DV I-incisor bar
Wistar	9.0	0.3	10.0	0.0	-1.3	-3.3
Female	9.4	0.8	10.0	0.1	-1.2	-3.0
Hooded	9.4	0.3	9.8	0.0	-1.2	-3.9
Sprague	9.0	0.7	10.1	0.1	-1.2	-3.9

311.12 THE COURSE OF EVOLUTION OF 15 TRAITS OF MAMMALIAN BRAINS, AS SHOWN BY COMPREHENSIVE NUMERICAL TAXONOMIC CROSS-ANALYSIS. J. I. Johnson, J. A. W. Kirsch*, and R. C. Switzer III. Anatomy Dept., Michigan State Univ., East Lansing, MI 48824; Dept. Organismic & Evolutionary Biol. and Mus. Comp. Zool., Harvard Univ., Cambridge, MA 02138; Pathology Dept., Univ. Tennessee, Knoxville, TN 37916.

We have previously derived a hypothetical tree of the lines of mammalian descent, based upon a comprehensive numerical taxonomic cross-analysis of primitive and derived states of 15 brain traits in 38 representative species (Kirsch, J. A. W. et al., Brain Beh. Evol. 22:70). In this communication we use this tree to describe the probable course of evolution, the changes that have taken place in phylogenetic history, in each of the 15 characters. Four characters have a common history, deriving from the ancestor of the placental branch: the development of a corpus callosum, the translocation of the facial nerve to a subtrigeminal position, the translocation of the ventral nucleus of the inferior olive from a lateral to a medial position, and the loss of oil droplets in retinal cones. Two characters, the occurrence of barrels in sensory neocortex, and gyrencephaly, proved to be multiply convergent, occurring in parallel in several disparate lines of descent. The remaining 9 characters each appeared in ancestors of one or another separate line of mammalian descent and characterize their related progeny as indicated below in parentheses. These include arteriovenous loops (in marsupials); widely separated thalamic optic terminals (in monotremes); course of the lateral olfactory tract through the accessory olfactory formation, and loss of the accessory formation (in gliro-unguiculates, and in insectivorous bats and old world anthropoid primates, respectively); the submerged position of the optic tract fibers in the tectum (in more derived therians); the doubling of oil-dropleted retinal cones (in Australian marsupials); the monolayering of olfactory mitral cells (in therians); the connection of dorsal cerebral neocortices by a fasciculus aberrans (in diprotodont marsupials); the loss of postcranial mechanosensory projections to cerebral cortex (in artiodactyls); and the ipsilateralization of oral projections to thalamus and cerebral cortex (in ferungulates). (Supported by NSF grants GB 43236 and GB 30783; NIH grants MH 10116, B-3249 and M-2786, and a grant from the Milton fund of Harvard Univ.).

311.13 COMPARATIVE MORPHOLOGICAL STUDY OF THE NEURONS OF THE SUBSTANTIA NIGRA (SN) OF VARIOUS MAMMALS. R. Marchand, L.J. Poirier and M. Giguère. Lab. Neurobiologie, Hôp. Enfant-Jésus, 1401, 18e rue, Qué. G1J 1Z4.

On the basis of morphological features, four distinct types of neurons were easily identified in the SN of the human, macaque, squirrel monkey, cat and rat. Previous reports have characterized various types of SN neurons rather by their sizes than by their cytological characteristics (Gulley and Wood, 1971; Schwyn and Fox, 1974 and others). Others (Rinvik and Grofova, 1970) have concluded to the presence of a single cell type in the SN. In the present account, based on Nissl preparations, compacta, reticulata, intermediary and globular neurons were distinctively characterized. These cell types are better differentiated in the primate brain (macaque and human) than in the cat and rat brains. The compacta type neurons are characterized in all the species studied by unevenly distributed, intensely stained, large clusters or trails of Nissl substance that do not constitute definite Nissl bodies. The relatively large and clear nucleus is eccentrically located and often bulges from the cell body at one point or another. They are of all shapes and sizes: 17-67um in the macaque; 13-60um in the cat and 10-29um in the rat. The reticulata type neurons are triangular or round. The perikaryon contains a fair amount of Nissl substance that is typically organized in definite Nissl bodies giving a tigroid appearance to the neurons. The nucleus is usually more centrally located than in the compacta variety. The cell body measures 13-58um in the monkey, 15-42um in the cat and 12-29um in the rat. In all species, there is much overlap between the sizes of the compacta and reticulata type neurons. Thus, it is obvious that such an overlap in sizes precludes that the diameters of these two main types of neurons may be used as a reliable criterion to characterize either type of cell. A detailed quantitative account dealing with cell sizes and absolute numbers is given in a companion paper. The intermediary type neurons are elongated, triangular, fusiform or, more rarely, polygonal in shape. The cytoplasm is chromatic with a few darker patches. The long cell processes are thinner than those of the compacta and reticulata type neurons. Finally, the globular neurons which are characterized by a high nuclear/cytoplasmic ratio are achromatic except in the rat where several of the globular neurons are lightly chromatic. Our data emphasize the heterogeneous nature of the cell population of the SN of various mammals. These different nigral cell types are most likely related to the various nigrofugal pathways subserving different functions. (Supported by the MRC (Canada) and FRSQ (Québec).

311.14 COMPARATIVE QUANTIFICATION AND DISTRIBUTION OF FOUR TYPES OF NEURONS IN THE SUBSTANTIA NIGRA (SN) OF THE MONKEY, THE CAT AND THE RAT. M. Giguère, L.J. Poirier and R. Marchand. Lab. Neurobiologie, Hôp. Enfant-Jésus, 1401, 18e Rue, Qué. G1J 1Z4.

Using cytological criteria as defined in a concurrent paper, the number and the size of four types of neurons (compacta, reticulata, intermediary and globular) of the SN were determined and their distribution studied in the brains of three species.

	MONKEY		CAT		RAT	
Compacta*	62,624+ (85)**	33.4++	22,323 (58)	31.1	9,925 (44)	17.2
Reticulata	8,772 (12)	28.2	12,409 (32)	25.3	3,122 (14)	20.0
Intermediary	2,112 (3)	25.8	709 (2)	24.8	3,227 (14)	16.0
Globular	few	14.2	2,925 (8)	14.2	6,259 (28)	12.1
Total Number	73,508		38,366		22,532	

+, mean number of cells; ++, mean longest diameter in um; *cell type; **, % in parentheses.

As shown in the table the total number of neurons of the SN which is much greater in the macaque than in the cat and the rat mainly involves the compacta type neurons. The reticulata type neurons are more abundant in the cat and the globular and intermediary type neurons in the rat. All four cell types have shorter mean diameters in the rat. The globular neurons which are smaller in all three species are few in number in the monkey SN proper.

The compacta type neurons occupy the most dorsal part of the SN over most of the rostrocaudal extent of this structure in the monkey. They are more abundant in the intermediate two fourths (71%) than in the rostral (12%) and caudal (16%) fourths of the SN. In the cat the compacta type neurons are more abundant in the caudal half of the SN (77% compared to 23% in the rostral half). In the rat the compacta type neurons are more abundant in the rostral half (74%) than in the caudal half (26%) of the SN. The reticulata type neurons which are more abundant in the rostral half of the SN are intercalated between the fibers in the most fibrous, rostrolateral part of the SN (subnucleus lateralis), and in the more dorsal part of the cerebral peduncle (subnucleus medialis). The intermediary type neurons are more specifically located in the ventromedial part of the rostral half of the SN. These peculiar features in different species must be taken into account when dealing with the interconnections of the SN. (Supported by grants from the Quebec FRSQ and the MRC of Canada).

311.15 EVOLUTIONARY ASPECTS OF CORTICAL ORGANIZATION IN THE DOLPHIN BRAIN. P.J. Morgane, A.M. Galaburda and M.S. Jacobs. Worcester Foundation for Expt. Biol., Shrewsbury, MA 01545, Dept. Neurology, Harvard Medical School, Boston, MA 02215 and New York U., New York, NY 10010.

The dual nature of the neopallium means that there are two neocortical moieties, one differentiated in stages away from the hippocampus and the other in stages away from the pyriform cortex. By successive waves of circumferential differentiation the growth rings of the cortex develop from allocortex to periallocortex to proisocortex to true neocortex. Using quantitative cytoarchitectonic analyses, we have traced the gradients of organization of the cortical formations of the dolphin brain, beginning in the paleo- and archicortical formations, across peripaleocortical and periarchicortical transitional areas, into the limbic lobe medially and insular division of the limbic lobe laterally. Peripaleocortical and periarchicortical formations represent the first incipiently laminated cortex and, as such, form the first growth ring of the neocortex which we term the primary stage of neocortical development. The second stage of neocortical development, the proisocortical stage, is represented by the limbic and insular cortices proper and shows a strong trend of differentiation in that emphasis is on the outer cortical lamina (Layers II, III and IV) instead of dominance of the inner cortical lamina (Layers V and VI) characteristic of the periallocortical areas. At this stage the outer cortical layers are becoming more cellular along with some regression of layer V pyramids. The third growth ring of the cortex, formed of two moieties, a paralimbic one and a parinsular one, has been defined by Sanides (1972) and others as the site of secondary (prokoniocortical) and supplementary sensory and motor representations in the brain. In the whale brain we see no true granularization trend extending outward and into the convexity cortices and, at the same time, the marked second layer accentuation of multiform and transitional pyramidal cells does not fade out in the cortical progressions to convexity cortex. The accentuated layer II present in convexity cortex of the dolphin, since it marks the periallocortical and proisocortical cortex stages, is another indication of arrested development of the dolphin brain at the parinsular/paralimbic stage of development. The accentuated layer II implies a protoneocortical mark indicating the originally prevailing layer I input of the axodendritic type. In following the cortical transitions outward in the dolphin brain the convexity cortex between both proisocortices shows no indications of hypergranular cores, i.e.,koniocortical development or gigantopyramidal development. It would appear that, like the hedgehog and bat brains, the dolphin brain has only reached the parinsular/paralimbic stages of neocortical evolution since these cortices appear to be dominant in the entire convexity cortex of the hemisphere. (Supported by NSF Grant BNS 82-42356).

BIOLOGICAL RHYTHMS: SUPRACHIASMATIC NUCLEUS

312.1 RETINAL GANGLION CELL PROJECTION TO THE SUPRACHIASMATIC NUCLEUS IN THE CAT AND RAT. C.A. Fuller, D.M. Murakami* and J.D. Miller, Division of Biomedical Sciences, University of California, Riverside, CA 92521

The suprachiasmatic nucleus (SCN) is the only nucleus within the hypothalamus to receive retinal ganglion cell (RGC) input. Photic information conveyed over this retinohypothalamic tract is responsible for synchronizing the 24-hour circadian rhythms observed in mammals. However, the results of recent studies suggest that in a variety of mammals there are several distinct populations of RGC. These distinctions are based on: 1) receptive field properties, 2) retinal distribution, 3) projection pattern and 4) morphology. Since these RGC populations have been extensively described in the cat, this species was chosen to determine which classes of RGC project to the SCN. After identification of the SCN (by X-ray and optic chiasm recording) injections of 20% HRP solution were made in the SCN either electrophoretically with small micropipettes, or by injecting 0.2 μl with a Hamilton syringe. Control injections of HRP were made into the optic tract. Retinas were dissected, reacted with DAB and counterstained with cresyl violet.

HRP labelled RGC were distributed across both retinas, with a tendency to be concentrated in the visual streak. HRP labelled cells were found in contralateral nasal, but not the temporal retina. In the ipsilateral retina, labelled cells were concentrated in the visual streak with an apparent even distribution across nasal and temporal retina. On the other hand, optic tract injections labelled cells in the ipsilateral retina with a predominant temporal distribution. Moreover, the control labelled cells of the ipsilateral nasal retina did not concentrate in the visual streak. The soma sizes of HRP labelled neurons in both peripheral retinas were small to medium in diameter (range: 12.0-19.0; X = 16.0 μ). Several cells with labelled dendrites showed a gamma cell pattern of arborization. The peripheral neurons in ipsilateral nasal retina were larger (range: 12.8-21.8; X = 17.8 μ) than cells labelled by control injections (range: 5-20; X = 12.1 μ). Cells labelled within and near the area centralis by SCN injections were significantly smaller (range: 10.8-15.8; X = 13.4μ) than those in the periphery. For comparison electrophoretic HRP injections were made into the SCN of rats. The labelled cells were medium to large in soma diameter (range: 9-21; X = 13.5 μ), with a Type I dendritic arborization pattern.

In the cat, cells labelled by SCN injection of HRP show characteristics of gamma cell soma size, dendritic arborization and retinal distribution. Preliminary evidence suggests that the largest RGC in the rat (Type I) project to the SCN, while the largest RGC in the cat (alpha) do not. These results may contribute to understanding the differences between cats, which lack overt circadian rhythmicity, and rats which demonstrate prominent rhythmic patterns. (Supported by NSF Grant BNS-792441 and PHS Grant RR-05816)

312.2 COMPARATIVE ANATOMY OF THE RETINO-HYPOTHALAMIC TRACT IN RODENTS. T. G. Youngstrom, H. E. Albers and A. A. Nunez. Psychology Dept. and Neuroscience Program, Michigan State Univ., E. Lansing, MI 48824 and Worcester Fnd. Exp. Biol., Shrewsbury, MA 01545.

A retino-hypothalamic tract (RHT) with projections to the suprachiasmatic nucleus (SCN) has been described in several species (Colman et al., Brain Res. 102:156-163, 1976; Moore, Brain Res. 49:403-409, 1973; Moore and Lenn, J. Comp. Neurol. 146:1-14, 1972). However, recent data suggest that there may be species specific differences with respect to pattern and symmetry of innervation of the SCN (Kita and Oomura, Brain Res. Bul. 8:249-253, 1982; Pickard, J. Comp. Neurol. 211:65-83, 1982). The development of very sensitive tracing techniques, such as horseradish peroxidase (HRP), has permitted the description of previously unknown or ill-defined neural pathways. Investigations of retinofugal pathways using unilateral injections of HRP have led to the identification of differences in retino-hypothalamic terminals in photoperiodic vs non-photoperiodic species of rodents. These differences in innervation may be the source of differential response to photic input (Pickard and Silverman, J. Comp. Neurol. 196:155-172, 1981). In this study we compared the RHT of mice (Mus musculus) and turkish hamsters (Mesocricetus brandti). These two species differ in their responses to changes in photoperiod. Injections of 30% horseradish peroxidase (HRP) within the vitreous of the eye were used. Brains were prepared for histology following a 24 hr. survival period using the method of Mesulam et al. (J. Histochem. Cytochem. 28:1255-1259, 1980) as modified by Pickard (personal communication). Frozen sections (20-50 microns) were reacted with tetramethyl benzidine. Selected sections were counterstained with pyronin Y and unreacted sections were mounted and stained with cresyl violet. The results confirmed the presence of a RHT in these two species. In the turkish hamster, retinal input extends throughout the SCN and appears approximately symmetrical as described in the golden hamster. Filling was denser in the rostral ipsilateral SCN becoming nearly symmetrical caudally with minimal filling dorsomedially. However, in the mouse, the density of HRP filling appeared asymmetrical, favoring the contralateral side throughout. As in the hamster, the dorsomedial portion of the mouse SCN lacked significant filling bilaterally. Highest concentrations of HRP reaction product were located in the dorsolateral, lateral and ventrolateral SCN. Retino-hypothalamic fibers approached the SCN in a pattern unique to the ipsilateral/contralateral relationship to the injected eye. An asymmetrical input to the SCN has been observed in the rat. The mouse retinal input differs from the rat, however, in that it is not restricted to the ventral half of the SCN. These and previous results using golden hamsters suggest that monocular symmetrical RHT input to the SCN may be a feature of photoperiodic animals.

312.3 SERIAL FINE STRUCTURAL ANALYSIS OF THE ORGANIZATION OF THE NEUROPIL OF THE SUPRACHIASMATIC NUCLEUS OF THE RAT. J.P. Card and R.Y. Moore. Departments of Neurology and Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, New York, 11794.

Neuropil within the suprachiasmatic nucleus (SCN) of the rat hypothalamus was subjected to serial fine structural analysis. Serial analysis was conducted in the coronal plane and was confined to the ventrolateral aspect of the intermediate third of the SCN rostrocaudal axis. Previous studies have established that this area represents a distinct subfield of the SCN in which neurons are both morphologically and chemically distinct and within which visual afferents establish highly localized and overlapping terminal fields.

Neuropil of the ventrolateral SCN is characterized by an extremely complex synaptology in which neuronal elements are extensively coupled via axo-dendritic, dendro-dendritic and axo-axonic synaptic contacts. Large numbers of dendrites are prevalent throughout the neuropil and receive synaptic input from a variety of morphologically distinct axon terminals. In addition, dendrites routinely form bundles in which dendro-dendritic synaptic contacts are a common feature. Dendrites also synapse upon spines of other dendrites or, more rarely, a dendritic spine will synapse with its parent dendrite. In rare instances, dendro-somatic synaptic contacts have been observed. Dendro-dendritic (and dendro-spinous) synapses are predominantly symmetrical in character with a focal aggregation of small (40nm) spherical synaptic vesicles immediately adjacent to the pre-synaptic membrane. Large granular vesicles (80-150nm) are commonly distributed throughout dendrites and sometimes occur in such large numbers that it is difficult to characterize the profile as dendritic without the benefit of serial sections. The ventrolateral neuropil is also characterized by conspicuous accumulations of axon terminals at the lateral extent of the SCN-optic chiasm interface. These terminals combine with small caliber dendrites and dendritic spines to form complex arrays of axo-dendritic, axo-spinous and axo-axonic synaptic contact. The majority of axon terminals contributing to these synaptic glomeruli conform morphologically to the category of optic-like boutons previously characterized by Güldner (1978) and therefore may represent retinal afferents.

These data demonstrate that SCN neurons influence the activity of one another via an extensive network of dendro-dendritic synapses and that there may be axo-axonic modulation of visual input to SCN. Each of these observations contribute to elucidation of the means through which the SCN integrates information and synchronizes neuronal activity in the control of circadian rhythmicity. Supported by NS-16304.

312.4 CIRCADIAN RHYTHMICITY IN MULTIPLE-UNIT ACTIVITY OF RAT HYPOTHALAMIC SLICE. T.G. Hedberg and M.C. Moore-Ede. Dept. Physiology and Biophysics, Harvard Medical School, Boston MA 02115.

Circadian rhythms in multiple-unit activity (MUA) have been demonstrated in the rat suprachiasmatic nucleus (SCN) when isolated *in vivo* in a hypothalamic "island" preparation (Inouye and Kawamura, *Proc. Natl. Acad. Sci. 6*: 5962, 1979). In order to investigate whether the mammalian SCN can generate persisting circadian rhythmicity in MUA when isolated from all sources of potential cyclic hormonal and afferent neural input, an *in vitro* brain slice preparation was developed with SCN-containing thin sections of rat anterior hypothalamus.

Young (150-200g) female, Long-Evans hooded rats were maintained for at least 2 months under a 14:10 LD cycle (L:0500-1900 hr) provided by a bank of cool white fluorescent bulbs with food and water provided *ad lib*. For each slice preparation, the animal was rapidly decapitated and the hypothalamus blocked out with a sterilized razor blade. A 300-400μ thick section containing SCN was then suspended in an enclosed chamber at the fluid/air interface of a slowly-moving, continually-replenished stream of oxygenated and glucose-supplemented Earl's balanced salts solution.

After ~90 mins of pre-incubation, sustained multiple unit discharges from SCN and regions immediately adjacent were recorded using an acrylic-insulated stainless steel electrode (tip diameter 10-15μ) at a uniform tissue depth of 100μ. Action potential amplitudes ranged between 15-30 μV *vs.* a peak background noise of 8-10 μV, and were counted at an amplitude window threshold of 15-20 μV. Counts were summed and stored in 5-min time bins via a computerized data collection system.

Initiation of slice preparations at different clock times revealed circadian rhythms in MUA discharge frequency in phase relationship with the previous photoperiod of the intact animal. In a series of 5 slice preparations whose survival times ranged between 18-62 hrs, MUA discharge frequencies alternatively decreased to a mean low of 22±30 counts/5 min during subjective night, and increased to a mean high of 274±30 counts/5 min during subjective day. Hypothalamic slices from rats which were previously maintained under an inverted 14:10 LD cycle (L: 19:00-09:00 hrs) demonstrated an inverted rhythm in MUA. We conclude that the rat SCN can generate circadian rhythmicity in MUA in the absence of all periodic inputs.

Supported by NIH grants NS13921, BRSG RR-05381 and GM29327; and AFOSR grant 83-0194.

312.5 CIRCADIAN RHYTHMS OF MULTIPLE UNIT ACTIVITY FROM HYPOTHALAMIC AND OTHER BRAINSTEM AREAS OF THE SQUIRREL MONKEY. Z. Boulos, D.E. Logothetis* and M.C. Moore-Ede. Dept. of Physiology and Biophysics, Harvard Medical School, Boston MA 02115.

Multiple unit activity (MUA) was continuously recorded in freely-moving squirrel monkeys for several weeks with the aim of comparing the temporal distribution of neural activity with that of other physiological and behavioral functions. Each monkey was chronically implanted with 4 bipolar electrodes aimed at various loci, including anterior, lateral, ventromedial and posterior hypothalamic areas, locus coeruleus, and midbrain reticular formation. The electrodes (25-125μ diameter, teflon-insulated platinum-iridium wire) were attached directly to 4 pre-amplifiers enclosed in a small metal box (33 x 33 x 10mm) which was fastened to the skull with dental acrylic. The signals were fed to external amplifiers through a commutator swivel, and from there to window discriminators and digital counters. An Apple II computer read the outputs of the counters and reset them at 5 min. intervals. Motor activity (head movements) and body temperature were also recorded.

Most of the brain areas exhibited clear circadian rhythms that entrained to daily light-dark (LD) cycles, and free-ran under continuous illumination with periods longer than 24 h. Ultradian oscillations superimposed on the circadian rhythms were also observed in several cases.

In all areas examined to date, the peaks of the circadian MUA rhythms coincided with those of the motor activity and temperature rhythms. Under LD cycles, this corresponds to the light segment of the cycle for these diurnally active animals. These results are comparable to those reported by Inouye and Kawamura (*Proc. Natl. Acad. Sci. U.S.A. 76*: 5961-5966, 1979) who found that in the rat, MUA rhythms from brain areas other than the suprachiasmatic nucleus also reached peak levels during the animals' active phase. For the nocturnal rat, however, this phase corresponds to the dark segment of daily LD cycles.

(Supported by NIH NS 13921 and GM 29327 and AFOSR 83-0194.)

312.6 CIRCADIAN RHYTHM IN ARGININE VASOPRESSIN CONCENTRATION IN THE RAT SUPRACHIASMATIC NUCLEUS. Marian S. Kafka, Marco A. Benedito,* Robert L. Zerbe,* and David M. Jacobowitz, NIMH, Bethesda, MD and E. Lilly and Co., Indianapolis, IN.

Although the suprachiasmatic nuclei (SCN) of the mammalian anterior hypothalamus are thought to function as a biological clock or pacemaker for circadian rhythms, the neurotransmitters participating in pacemaker activity are unknown. Such a neurotransmitter is expected to be intrinsic to the SCN and undergo a circadian rhythm. We investigated whether there was a circadian rhythm in arginine vasopressin (AVP), a putative transmitter which is intrinsic to the SCN[1]. In two experiments carried out 3 days apart, rats entrained for 3 weeks to a light:dark cycle (lights on 7H-19H), were enucleated. During the 67-91 hours after enucleation the rats were sacrificed, 5 rats at a time-point, every 4 hours, over 24-hour periods. The brains were removed, frozen, and the SCN microdissected. The concentration of AVP in each SCN was measured by radioimmunoassay[2]. In the two experiments the rhythms in mean vasopressin concentration were not significantly different by 2-way analysis of variance. Consequently, the data were combined. There was a circadian rhythm in AVP concentration with a peak at 14H and with the concentrations similar at all other time-points (statistics: one-way analysis of variance and Tukey's Test).

The rhythm in AVP concentration was reproducible in 2 studies carried out 3 days apart. The peak in the circadian rhythm in the AVP concentration occurs just after the midpoint of the increases in the circadian rhythms in SCN deoxyglucose uptake[3] and neuronal firing[4,5,6]. These processes of energy utilization and electrical activity are related to the pacemaking activity of the SCN[4]. It is possible that the circadian rhythm in AVP, a modulator or transmitter intrinsic to the SCN, plays a role in the SCN pacemaking function.

1) Moore, R.Y. Fed. Proc. (1983), In Press
2) Feuerstein, G. et al., Brain Res. Bull. 7:671-676 (1981)
3) Schwartz, W.J. et al., J. Comp. Neurol. 189:157-167 (1980)
4) Inouye, S.T. and Kawamura, H., J. Comp. Physiol. 146:153-160 (1982)
5) Green, D.J. and Gillette, R., Brain Res. 245:198-200 (1982)
6) Groos, G. and Hendriks, J., Neurosci. Lett. 34:283-288 (1982)

312.7 NICOTINIC ACTIVATION OF PHOTICALLY RESPONSIVE NEURONS IN THE RAT SUPRACHIASMATIC NUCLEUS. J.D. Miller, M.R. Crawford*, D.M. Murakami* and C.A. Fuller. Division of Biomedical Sciences, University of California, Riverside, Ca 92521.

The suprachiasmatic nucleus (SCN) of the hypothalamus has been identified as the locus of a primary circadian pacemaker in mammals. Damage to this nucleus results in the disruption of a wide range of endogenous circadian rhythms, whereas electrical stimulation of the SCN can produce phase shifts in circadian rhythms that are comparable to the effects of environmental zeitgebers. Further, neuronal activity of the SCN exhibits an endogenous rhythm, even in the isolated perfused tissue slice.

Visual stimuli appear to influence the activity of the SCN via the retino-hypothalamic tract. In addition, there is a high density of nicotinic receptors in the SCN. This study was designed to examine the relationship between photic and nicotinic stimulation of single neurons in the rat SCN.

Male albino rats (Sprague-Dawley, 300-350 g) were anesthetized with urethane and cannulated in the femoral vein. Standard electrophysiological techniques were used to record the activity of single units in the SCN with tungsten microelectrodes, according to the stereotaxic coordinates of de Groot. A fiber optics light source was used to apply photic stimuli directly to the eye. Neuronal activity was observed on an oscilloscope, monitored via polygraph, and recorded on magnetic tape for later analysis. An electrolytic lesion was made at the site of the cell recorded in each rat (10 u a/2 min). Subjects were perfused with saline followed by formalin. 40 micron coronal sections through the hypothalamus were subsequently stained with thionin.

Baseline firing rate of 7 SCN neurons examined to date was between 5 and 10 Hz, with action potential durations of approximately 2 msec and amplitudes of at least 50 u volts. Photic activation typically resulted in an enhancement of firing rate in SCN neurons, although inhibition was seen in one cell. In these photically responsive cells, nicotine (20 ug/Kg) caused a change in firing rate similar to the light response. Thus, photically excited cells were excited and photically inhibited cells inhibited. This nicotinic response was completely blocked by the nicotinic antagonist, mecamylamine (0.5 mg/kg). Further, mecamylamine also attenuated the effects of visual stimulation. Non-photically responsive cells (n = 5) of the anterior hypothalamus (non-SCN) were similarly not responsive to nicotine.

It appears that cholinergic afferents to the SCN may be of considerable functional importance in the control of SCN neuronal activity. Furthermore, this study suggests nicotinic modulation of the retinohypothalamic input to the SCN. (Supported by NSF Grant BNS-792441 and PHS Grant BRS RR-05816)

312.8 NEUROTRANSMITTER AND PEPTIDE LOCALIZATION IN RAT SUPRACHIASMATIC HYPOTHALAMIC NUCLEUS. R.Y. Moore and J.P. Card. Departments of Neurology and Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

The rat suprachiasmatic nucleus (SCN) can be subdivided into dorsomedial and ventrolateral components on the basis of cyto-architecture, neuronal morphology and the distribution of visual afferents. These subdivisions have been further delineated through recent immunohistochemical demonstrations of neurotransmitter and peptide localization. Retinal afferents distribute to the ventrolateral SCN (Moore and Lenn, 1972) and serotonin terminals are present in ventral SCN (Fuxe, 1964). These afferents are overlapped by a secondary visual input arising from neurons in the lateral geniculate nucleus and containing avian pancreatic polypeptide (APP)-like immunoreactivity (Card and Moore, 1982). Vasoactive intestinal polypeptide (VIP)-like immunoreactive neurons are localized in the ventral SCN and give rise to a dense axonal plexus which is largely restricted to SCN (Card et al., 1981). The present study describes the distribution of somatostatin (SS), vasopressin (VP), leu-enkephalin (L-ENK), neuronal peptide Y (NPY), molluscan cardioexcitatory peptide (FMRF) and glutamic acid decarboxylase (GAD) in the rat suprachiasmatic nucleus.

SS-containing neurons are concentrated in the dorsomedial SCN and give rise to a moderate plexus of fibers which extend throughout the nucleus, but are most dense ventrolaterally. VP neurons are also concentrated in the dorsomedial SCN and occur throughout the rostrocaudal axis of the nucleus. They elaborate an extensive axonal plexus which is largely confined to the limits of the SCN, but also extends dorsally into the periventricular nucleus. Neurons displaying GAD immunoreactivity are present in both SCN subdivisions and appear to project largely within the SCN. L-ENK containing neurons are found only in the rostral and dorsal SCN, but few immunoreactive axons occur within the nucleus. NPY and FMRF immunoreactivity is restricted to a dense terminal plexus in the ventrolateral SCN that is very similar to the APP immunoreactive axonal plexus.

These observations suggest two conclusions. First, the rat SCN contains a substantial number of local circuit neurons. This indicates that neuronal interconnection and integration is functionally important in the nucleus. Second, the subdivision of the SCN into dorsomedial and ventrolateral areas is supported not only by cytoarchitecture and the distribution of visual afferents, but also by the immunohistochemical organization of chemically distinct cell and fiber systems. This further emphasizes the possibility that these subdivisions are functionally significant in circadian rhythm generation and entrainment. Supported by NS-16304.

312.9 THE SUPRACHIASMATIC NUCLEI OF THE FETAL RAT: CHARACTERIZATION OF A FUNCTIONAL CIRCADIAN CLOCK USING ^{14}C-LABELED DEOXYGLUCOSE. S.M. Reppert & W.J. Schwartz. Children's & Neurology Services, Mass. General Hosp. & Harvard Med. School, Boston, MA 02114.

Glucose utilization (measured by the ^{14}C-labeled deoxyglucose method) has been found to provide an effective in vivo assay for the functional activity of the circadian clock located in the suprachiasmatic nuclei (SCN)(*Science* 197:1089; *J comp Neurol* 189: 157). We have used this technique to study the metabolic activity of the SCN in fetal rats and have found that (a) an entrainable circadian clock oscillates in the SCN during late fetal development, and (b) the maternal circadian system coordinates the phase of the fetal clock to the environmental lighting cycle.

Timed pregnant Sprague-Dawley rats were exposed to various environmental lighting regimens, outfitted with intra-atrial silastic catheters on gestational day 10, and injected intravenously with 145 µCi 2-deoxy-D-[1-^{14}C]glucose (s.a. 60 Ci/mole) at specified gestational ages and clock times. After 45 min, animals were sacrificed, fetal brains were removed and frozen, and serial 20 µm coronal sections were cut and autoradiographed.

Dams were exposed to a 12h:12h light:dark cycle until the 18th day of gestation, when they were placed and thereafter kept in constant darkness until they were injected on gestational day 21. Glucose utilization of maternal and fetal brains was high during mother's subjective day, low during mother's subjective night. A phase shift in the lighting cycle completed by the 6th day of gestation caused a corresponding shift in the rhythm of fetal SCN glucose utilization. When dams were blinded on the 1st day of gestation and the lighting cycle shifted, the fetal rhythms were synchronous with the circadian time of the blind mothers (out of phase with that expected had environmental lighting directly influenced the timing of the fetal clocks). The day-night metabolic oscillation was detectable in the fetal SCN from the 19th through the 21st days of gestation. The fetal SCN manifested high metabolic activity for a duration of 4 to 8 hrs during the subjective day on gestational day 21.

Thus, at a time in development well before the maturation of the neural mechanisms necessary for both the photic entrainment and overt expression of circadian rhythms, the mother acts as a transducer between the environment and the fetal brain, coordinating the phase of the developing circadian clock to her own clock time. Although data provided by indirect methods had suggested that the developing circadian clock might be functional during fetal life, the deoxyglucose method presently provides the only available means of directly investigating the function of the fetal circadian clock.

312.10 LITHIUM DELAYS THE PHASE OF RUNNING WHEEL RHYTHMS BUT NOT THOSE OF PINEAL MELATONIN OR SCN GLUCOSE METABOLISM IN RATS. D.L.McEachron*, D.F.Kripke*, F.R.Sharp,A.J.Lewy*,D.E.McClellan*] (SPON:S.Griffin).Dept. of Psych. Veterans Admin. Med. Center, San Diego, CA 92161.

To examine the effect of lithium (Li) upon circadian rhythms, 120 male rats were studied in two different, but related experimental protocols.

1) 90 rats were separated into 3 groups: 23 untreated(U),22 salt-fed(S)(diet was 5% NaCl), and 45 lithium-fed(L)(diet was 0.3% Li_2CO_3). All rats were housed on a 6:18 light-dark (LD) cycle (lights on from 8:00 am to 2:00 pm) for 12 days prior to the start of these diets and for 3 weeks of treatment. The rats were then sacrificed in counter-balanced order in one 24 hr period. Sixty-four minutes prior to sacrifice all S and 1/2 L rats received an IP injection of ^{14}C-2-deoxy-D-glucose, (2-DG; 7.8uCi/100 grams body weight) while all others received an equal volume of saline. Each animal was lightly anesthetized with methoxyflurane and then: 1. temperature was recorded with a rectal probe; 2. blood withdrawn for serum Li; 3. brain and pineal removed. The pineals were assayed for melatonin content, while the brains of the 2-DG rats were cut into 20u sections and the suprachiasmatic nuclei(SCN) analyzed for relative ^{14}C uptake using standard autoradiographic procedures. The data was analyzed by the single cosinor method (Nelson, et. al., Chronobiologia,6:305,1979)

The body temperature rhythm may have been phase-delayed by Li(L rats peaked at 9:20 am vs U at 4:20 am and S at 5:16 am) however, this could not be tested insofar as the data appeared bimodal and was not significantly fit by a single cosine. The fitted peaks for melatonin were 3:16 am for L, 3:24 am for U, and 2:20 am for S, while the peaks in the SCN's ^{14}C uptake were at 3:56 pm for S and 1:40 pm for L rats. The fitted peaks were not significantly different for either variable among the groups. Interestingly, the high ^{14}C uptake seen in the SCN during lights on did not diminish in either group until several hours after lights off.

2) 15 S and 15 L rats experienced the same conditions as above for 19 days and were then transfered to individual running wheel cages for two weeks. Least squares cosines were fit to each 24 hrs. of data and the vector means of the last 7 days' peaks from each animal were combined into group means for the S and L rats. The phase of the running wheel rhythm was significantly delayed in the L group (L peak at 1:45 am vs S peak at 11:00 pm; $F_{1,28}=4.49$, $p< 0.05$).

These results indicate that Li does not affect the rhythm of ^{14}C uptake in the SCN, but may affect another oscillator or the coupling between the SCN and running wheel rhythm in rats.

312.11 CIRCADIAN RHYTHMS IN RATS WITH SUBTOTAL LESIONS OF THE SUPRACHIASMATIC NUCLEUS. A. Robbins* and F. K. Stephan. Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306.

In the rat, total lesions of the SCN abolish or severely disrupt circadian rhythms, but even large partial lesions often appear to have little or no effect. These results could be explained by assuming that the organization of the circadian pacemaker is highly redundant so that only a small number of neurons are sufficient to sustain circadian rhythmicity. Alternatively, the pacemaker could exist in a specific locus within the SCN which must be included in effective lesions. These alternatives were examined by placing mechanical lesions of different sizes in various locations within the SCN and observing the effects on drinking rhythms. The damage ranged from 0-75% of SCN tissue destruction. Based on histological examination, rats were grouped as follows: no SCN damage (CON, N=8), unilateral damage only (UN, N=7), bilateral damage in non-overlapping regions (B-N, N=11), and bilateral damage in overlapping regions (B-O, N=10) of the SCN. Partial lesions had only minor effects on the entrainment of drinking rhythms to a light-dark cycle or on the response to phase shifts. In constant darkness, periodogram analysis indicated the presence of a statistically significant rhythm in the circadian range in all rats except one (B-O rat). However, B-O animals exhibited significantly greater variability in the daily acrophase of the freerunning rhythm than controls ($p<0.5$). The UN and B-N groups also showed an increase in variability although this effect failed to reach significance. In constant light (≈ 150 lux) 12 of 28 rats with lesions failed to show a statistically significant rhythm in the circadian range. This was observed in only 1 of 8 controls. No significant correlation was found between a specific location of damage and disruption of circadian rhythms.

These results suggest that there may not be a discrete location of pacemaker cells within the SCN. Conversely, they are consistent with the view of redundant and widely distributed organization of the pacemaking function within the SCN. Apparently, a small number of neurons are sufficient to maintain a nearly normal circadian drinking rhythm in the presence of a LD cycle. On the other hand, the loss of a substantial fraction of putative pacemaker cells, the disruption of coupling among such cells, as well as the destruction of efferent fibers could account for the observed destabilization of the drinking rhythm in constant conditions. Although redundancy is a common feature of the central nervous system, the present results are difficult to reconcile with the known neuroanatomical and neurochemical heterogeneity of the SCN.

312.12 EFFECTS OF SCN LESIONS ON HAMSTER RUNNING AND FEEDING RHYTHMS: IMMUNOHISTOCHEMICAL AND BEHAVIORAL ANALYSES. E.L. Gustafson* and L.P. Morin (SPON. G. Newman). Dept. of Psychology and Dept. of Psychiatry and Behavioral Science, SUNY, Stony Brook, NY 11794.

Lesions of the suprachiasmatic nucleus (SCN) disrupt the circadian rhythm of wheel running in golden hamsters (Rusak, 1977) In addition, it has been demonstrated that the SCN contains a wide variety of peptides and neurotransmitters. The goals of this experiment were 1) to measure changes in wheel running and feeding in SCN lesioned hamsters, and 2) to demonstrate the immunohistochemical evaluation of lesion placement.

Male golden hamsters were exposed to four different photoperiods (LD 14:10, LL, DD, LD 6:18, respectively) for six weeks per photoperiod. After entrainment to and during LD 14:10, the SCN of each hamster was given a radiofrequency lesion. Following the experiment, all animals were perfused and the brains were processed for immunohistochemical and Nissl analysis of lesion placement. Wheel running and feeding data were analyzed using power spectrum and periodogram programs.

Animals in which the SCN lesions proved to be complete showed arhythmic wheel running under all lighting conditions. Several animals in which the rostral 80-90% of the nucleus was destroyed remained entrained to LD 14:10 after the lesion, but showed complete disruption of periodicity during constant conditions and during LD 6:18. Immunohistochemical analysis of these lesions showed no remaining vasopressin fibers or cell bodies, while still showing the presence of other peptides, most notably vasoactive intestinal polypeptide. Average daily activity consistently increased during DD as compared to LL, regardless of lesion extent. The ultradian nature of feeding behavior was largely undisturbed, but SCN lesions abolished specific characteristics of hamster feeding which are associated with early subjective night.

Immunohistochemical analysis of intact hamster SCN suggests that it is larger than revealed by Nissl analysis. The histochemical approach has also proven extremely useful in ascertaining the amount of SCN surviving after partial lesions, as Nissl analysis very rarely indicated the intact portions of the nucleus in this study. Our results suggest that it may soon be possible to associate the presence or absence of a particular peptide with rhythmic events.

Supported by NICHD grant HD16231.

312.13 DAILY RHYTHMS IN LUTEINIZING HORMONE RELEASE, INCORPORATION OF ^{35}S FROM METHIONINE INTO CEREBRAL PROTEIN AND DRINKING ACTIVITY IN THE RAT: EFFECTS OF LESIONS OF THE SUPRACHIASMATIC NUCLEI OR THEIR 5-HYDROXYTRYPTAMINE-CONTAINING AFFERENTS.
Clive W. Coen* (spon: W.F.Collins Jr.)
Section of Neurosurgery, Yale University School of Medicine, New Haven, CT 06510.

The suprachiasmatic nuclei (SCN) represent the densest concentration of 5-hydroxytryptamine (5-HT) terminals in the forebrain and also the site of a mechanism involved in the generation of circadian rhythmicity. Lesions of the SCN result in a failure of the daily luteinizing hormone (LH) surge in oestrogen-treated ovariectomized rats. This LH surge is associated with a daily peak in the incorporation into trichloroacetic acid precipitable protein of ^{35}S, administered as ^{35}S methionine, within the amygdala, putamen, thalamus, preoptic area and region of the median eminence. This rhythm of incorporation is eliminated by lesions of the SCN; loss of the 5-HT projections to the SCN, induced by the infusion of 5,6-dihydroxytryptamine into the dorsal and median raphe nuclei, has no effect on the pattern of incorporation or, in individually housed animals, on the normal occurrence of the surge. Although the nocturnally biased rhythm of drinking is lost following removal of the SCN, this rhythm is unaffected by lesions of the 5-HT projections from the raphe nuclei or by treatments with p-chlorophenylalanine (PCPA) or p-chloroamphetamine (PCA) resulting in a severe depletion of 5-HT throughout the brain. Identical treatments with PCPA or PCA do, however, block the daily LH surge in oestrogen-treated ovariectomized rats (Coen & MacKinnon, J. Endo. 82: 105, 1979). Recent studies (Coen et al., Neuroscience 8: 583, 1983) indicate, however, that the capacity of the latter compounds to prevent the LH surge and the restoration of the surge in PCPA-treated animals by administration of the 5-HT precursor, 5-hydroxytryptophan, are primarily related to the respective depleting and restoring effects of these drugs on the hypothalamic concentration of adrenaline rather than 5-HT.

These results indicate that the indispensable role of the SCN in various endocrine, metabolic and behavioural rhythms is probably unrelated to the 5-HT innervation of these nuclei.

312.14 THE EFFECTS OF PINEALECTOMY ON THE RUNNING ACTIVITY RHYTHMS OF RATS WHOSE SUPRACHIASMATIC NUCLEI HAVE BEEN SPLIT. Yanovski, J.*, Rosenwasser, A, and Adler, N. Psychology Department, University of Pennsylvania, Philadelphia, Pennsylvania, 19104

A number of authors have suggested that the expressed circadian rhythms of rodents may be the result of the output of a multioscillator system whose substituent oscillators are held in synchrony through mutual coupling relationships. We have attempted to study the importance of neural and hormonal factors in maintaining coupling within the circadian oscillatory system of the female Sprague Dawley rat.

Two years ago, some of us (Rosenwasser, Eng and Adler) reported that unilateral eye removal combined with a knife cut procedure that severs the direct neural connections between the two suprachiasmatic nuclei (SCNs) without damaging either (splitting of the SCNs) produce severe disruptions in free-running rhythms when animals are placed under constant light (LL) or constant dark (DD), but not under light/dark cycles. We now report that pinealectomy combined with splitting of the SCNs can also lead to complete breakdown in running activity rhythms of female rats when they are placed under constant conditions.

Experimentally naive female Sprague Dawley rats were subjected to one of three surgical procedures: pinealectomy plus sham splitting of the SCNs, actual splitting of the SCNs plus sham pinealectomy, or both pinealectomy and splitting of the SCNs. Running activity rhythms of all three groups of rats were examined under conditions of entrainment to LD 12:12, 6 hour phase delays, LL and DD.

All groups were able to respond to entrainment and phase delay conditions normally. However, several of the rats who received both pinealectomy and splitting of the SCNs demonstrated arrhythmic running patterns under LL and DD, similar to those seen in animals who have undergone bilateral ablations of the SCNs. The other experimental groups expressed normal free-running rhythms under constant conditions.

These results are interpreted in terms of a role for melatonin as a promoter of coupling within the circadian multioscillator system of the rodent. It is possible that other hormones may also act to maintain coupling relationships; the importance of such hormones may be demonstrable in the split SCN animal.

312.15 HYPOTHALAMIC CIRCUITS AND FREE-RUNNING CIRCADIAN RHYTHMS.
M. H. Brown* and A. A. Nunez. Department of Psychology and Neuroscience Program, Michigan State University, E. Lansing, MI 48824.

Total or nearly total lesions of the suprachiasmatic nucleus (SCN) abolish free-running circadian rhythms in a variety of behaviors. Anatomical studies (Stephan et al., Neurosci. 6:2625, 1981) have shown that SCN neurons send efferent projections rostrally, caudally, dorsally, and laterally. Surgical isolations of the SCN which cut all of these projections abolish rhythms in drinking, activity, sleep, and brain temperature (Stephan & Nunez, Behav. Biol. 20:1, 1977). Cuts which sever all of the SCN efferents except those projecting rostrally also abolish rhythms. More selective cuts which sever only fibers projecting rostrally, caudally, or laterally do not abolish behavioral rhythms. These findings indicate that: (i) the SCN is necessary for the generation of normal rhythms, (ii) rhythms are mediated by SCN efferent projections, (iii) rostral connections alone are not sufficient to maintain rhythms. The evidence indirectly suggests that either there is functional redundancy in the circadian system or behavioral rhythms are mediated by dorsal or dorso-lateral efferent projections from the SCN. These conclusions are tentative, however, since animals with cuts severing lateral or rostral projections alone were never observed in constant environmental conditions.

To further investigate the role of SCN efferent projections in the generation of behavioral rhythms, male rats housed in constant light were given either horizontal (HZ) cuts aimed dorsal to the SCN or bilateral parasagittal (PS) cuts lateral to the SCN. Drinking spout licks and wheel running activity were measured, plotted, and analyzed by a computer. In all cases, the HZ cuts damaged the dorsal part of the SCN and resulted in arrhythmicity for 7 to 10 days after surgery. The PS cuts did not damage the SCN and did not disrupt rhythms although one PS rat showed a change in period after surgery. Histological examination revealed that the cut in this rat resulted in damage to the optic chiasm which may account for the change in period.

Results of this and previous experiments suggest that (i) there is functional redundancy in the hypothalamic circuitry that mediates behavioral rhythms, (ii) SCN efferent projections which leave the nucleus in a dorsal direction may be more important than other projections in the generation of behavioral rhythms.

312.16 CIRCADIAN RHYTHM OF LUMINANCE DETECTABILITY IN THE RAT: INDEPENDENCE FROM SCN PACEMAKER.
M. Terman and J. Terman*. Dept. of Psychology, Northeastern Univ. Boston, MA 02115.

A signal detection procedure yielded continuous psychophysical records of visual sensitivity for Long-Evans rats given lesions of the suprachiasmatic nuclei and maintained under freeruns and skeleton photoperiod entrainment. An animal initiated a trial by positioning its head for observation of a 0.5-sec LED flash in the scotopic threshold range. Correct detections (hits and correct rejections in a two-choice task) were reinforced by hypothalamic brain stimulation, revealing circadian rhythms of differential behavior indexed by the bias-free sensitivity parameter, d'. Feeding and drinking behavior were monitored concurrently.

All animals showed reliable circadian oscillations in visual sensitivity. Under DD freeruns, periods ranged from 23.75 to 24.45 h. Under skeleton photoperiods, two 15-min pulses of broad-spectrum fluorescent light, separated by 12 h, were presented each day. (Pulse-induced transient sensitivity decrements and readaptation intervals were excluded in analyses of the daily rhythm.) Intact animals showed freerunning and entrained ingestive rhythms similar to the sensitivity rhythm in period and subjective night. SCN animals showed arrhythmic ingestion patterns, with occasional cases of greatly dampened residual drinking rhythms.

Under entrainment, the two daily light pulses assumed a consistent relation to the sensitivity waveform, one positioned within the ascending phase, and the other within the descending phase. Neither pulse could be interpreted as initiating the daily increase or decrease in sensitivity, since those trends were already underway at the time of pulse presentation. The daily nadir in sensitivity occurred within 2-5 h of the "morning" pulse, a phase similar to that of the circadian burst in rod outer-segment disc shedding, as observed under LD. In response to phase advances or delays of the skeleton photoperiod, some SCN animals showed corresponding gradual daily adjustments, while others showed a transient fractionation of the waveform and regrouping at the newly-appropriate phase. (One animal showed a spontaneous phase jump, requiring 14 days for re-entrainment.) Presentation of a single 1-h light pulse late in the freerunning high-sensitivity phase induced a phase advance of 2-3 h, and an early pulse induced a phase delay of similar magnitude.

We conclude that the visual sensitivity rhythm exists independently of the SCN pacemaker system. It is unperturbed by lesion-induced arrhythmicity in feeding and drinking, and shows the main features of photic phase control found for motor activity rhythms in rodents with intact SCN.

Supported by NIMH Grant MH 27442.

312.17 THE CIRCADIAN TIMING SYSTEM: A SECOND OSCILLATOR.
Kevin Maguire*, Dr. Richard Gold, Anita Sylven*
Psychology, Amherst, MA, University of Massachusetts, 01003

The suprachiasmatic nucleus (SCN) of the hypothalamus has been postulated as a circadian oscillator. Large bilateral SCN lesions result in the abolition of circadian rhythms for feeding, drinking and running. However, these observations typically begin 6-8 weeks after surgery and employ relatively massive lesions that destroy more than the SCN. In the present study, post-operative rhythms of food, water, and wheel running were observed immediately following discrete bilateral SCN lesions. Nine one-year-old F-344 female rats were individually housed with ad lib access to Purina lab powder, tap water, and a Wahmann running wheel under a 12:12 hr. dim light/dark photo period (5 foot candles). Bilateral SCN lesions were made via a platinum electrode using a 1 milliamp anadul current for 10 seconds. Virtually no wheel running was observed post-operatively until food was withheld. Under food deprivation non-circadian running was observed. The response to cold stress will also be recorded. However, the circadian rhythms of eating and drinking resumed within hours after surgery. Under constant light conditions, a free running circadian rhythm for eating and drinking, but not running were observed. If the SCN were the sole pacemaker, destruction of this oscillator would have to lead to total arhythmicity. However, here that was not the case. A second oscillator, perhaps in close proximity to the SCN, is implicated.

313.1 EXOGENOUS SEROTONIN PHASE-SHIFTS AND SYNCHRONIZES FREE-RUNNING CIRCADIAN LOCOMOTOR RHYTHM IN APLYSIA. B.Jahan-Parwar. Worcester Foundation for Experimental Biology,Shrewsbury, MA 01545.

Locomotion in Aplysia californica displays a diurnal circadian rhythm which appears to be timed primarily by a circadian oscillator system located within the eyes (Jacklet,1969; Strumwasser, 1974;Lickey et al.,1977). The mechanism by which this ocular circadian oscillator system transmits timing cues to locomotor control system is unknown. Several lines of evidence suggest that serotonin may be involved: 1) serotonin is synthesized in the eye and can phase shift and entrain the ocular oscillator (Corrent and Eskin,1982);2) endogenous serotonin can be measured in Aplysia hemolymph with HPLC and electrochemical detection method (unpublished data); 3) serotonin serves as neurotransmitter in Aplysia CNS (Gerschenfeld et al.1976); 4) a brief serotonin injection arouses Aplysia and induces locomotor activity (Mackey et al.,1981;Palovcick et al.,1982). 5) serotonin has been implicated in control of rest activity states in other animals (Flicker et al.,1981). As the first step in testing this hypothesis, we examined the effect of exogenous serotonin on freerunning circadian locomotor rhythm in Aplysia. Locomotion was continuously measured by placing Aplysia in individual activity wheels (modified from Jacklet,1972) and monitoring wheel rotation using optic couplers and a laboratory computer. Aplysia (N=6) initially entrained to a LD, 12:12, were chronically implanted with a catheter into hemocoel, and were exposed subsequently to constant darkness. Beginning with the onset of each projected dark cycle, each subject received a 6-hour long infusion of filtered seawater at the rate of 0.4 mL/hr. The 10^{-7} M/kg serotonin. The other three served as control subjects. Prior to implantation, the locomotor activity of all subjects was synchronized by the light cycle. Following implantation, the control subjects displayed typical freerunning locomotor rhythm with a period within the circadian range and peak activity occurring during the projected light cycle. The experimental subjects also displayed a circadian locomotor rhythm. The peak activity in these, however, was shifted into the projected dark cycle and occurred during serotonin injection. These results, while not conclusive, support the hypothesis that serotonin is involved in mediation of circadian timing to locomotor control system. Since the cellular organization of locomotor control system, including locomotor command system, has been extensively delineated in this laboratory, we are in a position to begin elucidating how serotonin regulates excitable properties of locomotor control system. Supported by PHS grant NS-12483.

313.2 ROLE OF cGMP IN MEDIATING THE EFFECT OF LIGHT ON A CIRCADIAN RHYTHM FROM THE APLYSIA EYE. A. Eskin, J. Takahashi, M. Zatz, and G. D. Block. Biology Dept., Univ. of Houston, Houston, TX 77004; NIMH, Bethesda, MD; Univ. of Va., Charlottesville, VA.

We are attempting to identify the events involved in carrying photoentrainment information from the environment to a circadian oscillator that is in the isolated eye of Aplysia. Previous results of these studies indicate that light pulses phase shift the rhythm from this eye by causing photoreceptor potentials that depolarize membrane potentials. Also, synaptic potentials do not appear to be involved in the effect of light on the eye rhythm.

An investigation of the role of cGMP in the phase shifting effect of light began when we discovered that 10 min and 50 min light pulses increased cGMP by 52% and 28% respectively in intact eyes. Light had no significant effect on cAMP. The idea that light caused phase shifts by increasing cGMP was tested by comparing the effects of light and Br-cGMP (8-bromo cGMP) on the rhythm and by an additivity experiment with treatments of light plus Br-cGMP. Br-cGMP (2mM, 6 hr) precisely mimicked the effect of 6 hr light pulses. Phase response curves obtained using Br-cGMP or light were indistinguishable from one another. In addition, the effect of Br-cGMP and light on the rhythm was non-additive. The phase shift produced by light plus Br-cGMP was not significantly different from the phase shift produced by either treatment alone. The specificity of the phase shifting effect of Br-cGMP was explored by exposing eyes to 8-bromo 5' GMP or 8-bromo cAMP. 8-bromo 5' GMP did not phase shift the rhythm. 8-bromo cAMP did phase shift but it did not mimic the effect of Br-cGMP. Br-cAMP produced delay phase shifts at a phase where Br-cGMP produced advance phase shifts.

The involvement of membrane potential depolarization in phase shifting by light and the fact that Br-cGMP increased the frequency of spontaneous nerve impulses from the eye in a manner similar to light led us to ask if Br-cGMP phase shifted through depolarization. Depolarization appears to mediate the effect of Br-cGMP on the rhythm because combining a low Na^+(12mM) treatment with Br-cGMP completely abolished the phase shift normally produced by Br-cGMP. The results presented here taken together with previous data indicate that light phase shifts the eye rhythm by increasing cGMP which then causes a depolarization. These steps do not appear to be occurring in the organized layer of microvillous photoreceptors beneath the lens because the membrane potential of these photoreceptors was not depolarized by Br-cGMP. Apparently, light and cGMP are acting on the circadian oscillator in the eye by their effects on other photoreceptors and higher order neurons. Supported in part by NIH Grant BRSG SO7 RR 07147-10 and NSF grant BNS 82-16756.

313.3 PROTEIN PHOSPHORYLATION ASSOCIATED WITH PHASE SHIFTING BY SEROTONIN IN THE EYE OF APLYSIA. D. Crommie*, A. Eskin+ and S. Arch. Biological Laboratories, Reed College, Portland, OR 97202; +Biology Department, Univ. Houston, Houston, TX 77004.

A variety of physiologic and pharmacologic studies have demonstrated that the circadian rhythm of compound action potentials in the isolated eye can be phase shifted under in vitro conditions. Among the effective agents, serotonin (5-HT) has attracted particular attention because it affords the opportunity to probe biochemical processes involved in the oscillating mechanism. Evidence for its importance as an endogenous phase-shifting agent can be illustrated by noting that 5-HT is present in the eye, that it induces cyclic adenosine monophosphate (cAMP) synthesis, and that 8-benzothio cAMP and forscolin mimic the phase-shifting action of 5-HT. Hence, investigation of the consequences of elevated cAMP levels represents a logical next step in the molecular specification of this phase-shifting pathway. Certainly the best characterized role for cAMP in intracellular regulation is kinase activation and the consequent alteration of the phosphorylation status of proteins. We, therefore, employed "back-phosphorylation" assays on eyes exposed to 5-HT for different durations and at different phase points in the circadian cycle. Eyes were dissected from freshly killed animals at a fixed time for each experiment. They were then placed in constant darkness and held until control or experimental treatment. The principal experimental group was treated with two changes of $5X10^{-6}$M 5-HT for the 6 hr period during the cycle at which a maximal phase-advance is expected. Three types of controls were prepared as well: 1) Eyes taken through the same procedures at the same phase but not exposed to 5-HT; 2) Eyes treated as above except with a 20 min exposure to 5-HT, a condition that does not result in a phase shift; 3) Eyes exposed to 5-HT at a phase point when 5-HT does not produce a phase advance. Following treatment, the eyes were frozen and stored at -70 C until preparation for phosphorylation. Back phosphorylation in the presence of added cAMP was performed on pooled homogenates of the eyes in the various treatment groups. After electrophoresis of homogenate samples on SDS-polyacrylamide gels, autoradiograms were produced. These revealed 11-15 bands of phosphorylated proteins. Most of these bands corresponded to Coomassie-stained bands visualized on the same gels. Of the phosphorylated species two low molecular weight proteins (3,900 and 3,175 dal.) appeared to be strongly phosphorylated only when 5-HT is present during the entire 6 hr period when phase advance is maximal. On the basis of centrifugation studies these small proteins appear to be membrane associated.

313.4 A PLASTIC BEHAVIORAL EXPRESSION OF CEPHALIC CIRCADIAN PACEMAKERS IN SPLITBRAIN CRAYFISH. D.E. García-Díaz* B. Barrera-Mera (SPON: J.A. Roig). Depto. de Farmacología Fac. de Medicina,U.N.A.M., Depto. de Neurociencias, Centro de Investigaciones en Fisiología Celular, U.N.A.M. México.

To date physical stimulation (photical, thermal, mechanical and electrostatic fields effect), the effect of hormones and also complex experimental manipulation, have been used to explore the plastic properties of interacting circadian pacemakers in metazoan organisms. While driving the retinal sensitivity in crayfish, left and right circadian pacemakers behave as a single one or compound circadian pacemaker (B.Barrera-Mera, et.all. Brain. Res. Bull.5,667.1980). But as in intact hamsters (G.E.Pickard, F.W.Turek, Science 215,1119.1982), they show clear unability to free runn simultaneously in splitbrain crayfish. We hypothesized that the simple interruption of circadian pacemakers reciprocal connections in these preparations, would render an excellent condition to inquire into the plastic properties of paired circadian oscillating structures. In contrast with the selfsustained circadian rhythm in the electroretinogram (ERG) amplitude of intact crayfish, ERG rhythm becomes damped in splitbrain crayfish. In these conditions, left and right damping of ERG oscillations follows a different, i.e. alternant (N= 11) or symmetrical (N= 16), time course as compared with one another side. But a drastic reversion of damping was obtained once the contralateral test light stimuli were strongly diminished or suppressed at all. Given the redundant processing of pacemaking information coming from bilaterally positioned circadian pacemakers to the central nervous system in splitbrain crayfish, photodependent damping of ERG rhythm revealed a plastic potential of central (cephalic) circadian pacemakers. The possibility that strong but reversible central inhibitory influence acting upon the protocerebral circadian pacemakers is considered; as if such pacemaking structures were developing non simultaneous functional activity after their drastic reciprocal disconnection.

313.5 NEURAL REGENERATION AND INTERNAL COUPLING IN THE CIRCADIAN SYSTEM OF THE COCKROACH. Terry L. Page, Department of General Biology, Vanderbilt University, Nashville, TN 37235.

The pacemaker that regulates the circadian rhythm of activity in the cockroach Leucophaea maderae is composed of two oscillators, one located in each optic lobe of the brain. The output signal of each oscillator has 2 functionally distinct destinations. The first is to midbrain structures that regulate activity levels. The second is to the pacemaker in the contralateral optic lobe. Previous results have shown that after optic tract section or optic lobe transplantation the neural connection from the pacemaker to the structures in the brain that control activity regenerates.

In present experiments the possibility that the pathway that couples the two oscillators also regenerates was investigated. One approach was to examine the effects of transplanting a single optic lobe between individuals whose freerunning periods differed by more than 1 h. The assumption was that regeneration of the coupling pathway would cause a substantial change in the freerunning period of the host activity rhythm. In 13 transplants there was no instance of a significant change in period ($\overline{\Delta\tau}$=0.1±0.16 h). However, there was no indication either of the appearance of a second activity component as might be expected if the transplanted lobe had regenerated its output connections to the midbrain, but had not become coupled to the contralateral pacemaker. To confirm that regeneration had occured the optic tract of the host optic lobe was cut. Of the 13 animals 7 exhibited a clear rhythm of activity beginning 1-3 days after surgery with a period near that expected for a rhythm driven by the donor's pacemaker. Phase differences between pre- and post-operative rhythms were variable ranging from 2-11 h. In a second series of experiments the transmission of entrainment information from one compound eye to the contralateral pacemaker was investigated. One optic tract was cut and allowed to regenerate in 8 animals. The animals were then placed in a 23 h light cycle (LD 10:13). All animals entrained normally. After 2 weeks the optic nerve of the intact optic lobe was cut isolating that pacemaker from its compound eye. The 6 animals that survived surgery began to freerun in the LD cycle. In no case was there evidence of an entrained (23 h) component to the activity rhythm. The results indicated that entrainment information transduced by the eye of the regenerated optic lobe was not reaching the contralateral pacemaker. After 4 weeks the optic tract of the intact lobe was cut. In 5 of the 6 animals a stably entrained 23 h rhythm was apparent within three days of surgery. When placed in DD a freerunning rhythm persisted in all 5 animals. The results of these experiments suggest (1) the coupling pathway between the optic lobes does not regenerate after optic tract section or optic lobe transplantation, and (2) an intact optic lobe can suppress the expression of an optic lobe that has regenerated its output connections.
(Supported by PHS-NIH Grant GM30039)

313.6 EFFECT OF POSITIVE AND NEGATIVE ATMOSPHERIC IONS ON HIPPOCAMPAL PYRAMIDAL NEURON RESPONSIVENESS TO SEROTONIN. M.J. Dowdall* and C. de Montigny (SPON: B.O. Dubrovsky). Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Québec, Canada H3C 3T8.

Exposure to negative air ions has been reported to decrease brain serotonin (5-HT) content in the rat (Science 210:652, 1980) and to reverse "hyperserotoninergic anxiety" in humans (Neurosci. Abst. 8:76.9, 1982). The present studies were undertaken to determine the effect of long-term exposure to positive or negative ions on the responsiveness of forebrain neurons to 5-HT.

Three groups of male Sprague-Dawley rats (initial weight 100 g) were studied: a control group and groups exposed to negative or positive ions for 21 days. Ions were generated by a wire bearing a 4.0 kV potential, at a concentration of approximately 1.5 X 10^6/cc. All rats were exposed to the same light:dark cycle (daylight). Unitary extracellular recordings from pyramidal neurons of the CA_1 and CA_3 regions of the dorsal hippocampus were obtained under urethane anesthesia (1.25 g/kg, i.p.) with five-barrelled micropipettes. Side barrels were filled with 5-HT (0.5 mM in 200 mM NaCl; pH 4), norepinephrine (NE) (20 mM in 50 mM NaCl; pH 4), and acetylcholine (ACh) (20 mM in 200 mM NaCl; pH 4). Silent or slowly discharging neurons were activated to 8-12 Hz with a small current (1-5 nA) of ACh. Responsiveness to 5-HT and NE was assessed using the $I.T_{50}$ method (i.e. current x time required to achieve a 50% inhibition of firing rate from baseline).

Spontaneous rate of neuronal firing and responsiveness to ACh and NE were comparable in all three groups. Consistent with a previous report from our lab (Neurosci. Abst., 6:280.5, 1980), a diurnal variation of responsiveness to 5-HT was detected in the control group, sensitivity being minimal in the morning and maximal in the evening. Exposure to either negative or positive ions disrupted this circadian rhythm. In rats exposed to negative ions, responsiveness was equivalent throughout the day to that found in the evening in the control group, whereas in rats exposed to positive ions, responsiveness was similar throughout the day to that found in the afternoon in the control group. The mean $I.T_{50}$ value for the whole day was therefore significantly smaller in the group exposed to negative ions than in the control group; there was no difference between mean $I.T_{50}$ values of the group exposed to positive ions and of the control group.

The increased sensitivity of forebrain neurons to 5-HT and/or the reduction of diurnal fluctuations of responsiveness to 5-HT might be related to the behavioral effects of exposure to negative ions.

313.7 CIRCADIAN PHOTORECEPTION IN RODLESS (rd) MICE. D. J. Hudson* and M. Menaker. (SPON: M. Lickey). Institute of Neuroscience. University of Oregon, Eugene, OR 97403.

The photoreceptors that supply environmental information to the circadian system of mammals are located within the eye, however the properties of circadian photoreceptors differ markedly from the properties of those involved in vision. In our attempts to characterize and identify the photoreceptors within the retina that supply information to the circadian system, we have used the C57BL/6J mouse carrying the mutant allele rd. Mice homozygous for the allele rd lack any organized photoreceptor outer segments after about 6 weeks of age, and suffer obvious visual deficits.

We have measured the "circadian photosensitivity" of homozygous rd mice and their heterozygous siblings with normal retina, by measuring the phase shifts in their freerunning locomotor rhythms produced by light pulses of varying intensity. Mice in individual running wheel cages were tranferred from light cycles into constant darkness and exposed to monochromatic light pulses (515 nm) of between 2 X 10^{11} and 9 X 10^{15} photons · cm^{-2} · sr^{-1} at 80 days of age. This is several weeks after the disappearance of all photoreceptor outer segements and the loss of all rod nuclei, though some cone nuclei survive for much longer periods. Light pulses were given 4 hours after locomotor onset, a time previously shown to produce maximal delaying phase shifts.

Loss of rods and most cones renders the homozygous rd mice only slightly less photosensitive than their heterozygous siblings. The fluence-response relationships for the two groups are shown in Fig. 1.

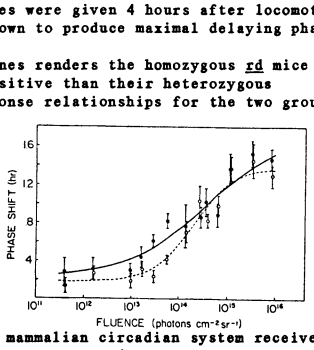


Figure 1. Fluence-response relations of homozygous rd (open circles) and heterozygous rd mice. The "best-fit" rectangular hyperbola is shown for each group (dashed line: homozygotes; solid line: heterozygotes).

These data suggest that the mammalian circadian system receives its major photic input not from rods but from either cones or cells in the eye which have not yet been identified as photoreceptive.
(Supported by the Medical Research Foundation of Oregon and NIH HD13162.)

313.8 BICUCULLINE SELECTIVELY BLOCKS LIGHT INDUCED PHASE DELAYS IN THE CIRCADIAN RHYTHMS OF HAMSTERS. M. R. Ralph* and M. Menaker. Institute of Neuroscience, Dept. of Biol., University of Oregon, Eugene, OR 97403.

Although several chemical factors have been shown to affect various properties of circadian rhythms, few neuropharmacological agents have been found that mimic or block the effect of light pulses on circadian systems. Using known specific antagonists and agonists of GABA, we are investigating the possible involvement of a GABAergic mechanism in the light input pathway. We have found that bicuculline, a potent GABA antagonist, blocks the effect of phase delaying light pulses on the hamster locomotor rhythm, but does not block the effect of phase advancing pulses.

Male golden hamsters (10-12 weeks of age), housed individually in running wheel cages in light tight boxes, were entrained to a 14:10 LD cycle for 7 days, and then released into constant darkness (DD). After 7 days in DD, each animal was given a 15 minute pulse of monochromatic light (515 nm.; total fluence = 3.0 x 10^{14} photons·cm^{-2}·sr^{-1}) calculated to produce a half maximal phase shift at either phase advance (CT 18) or phase delay (CT 13.5) time points. At both time points, experimental groups (N=13) received an injection of bicuculline (4.0 mg/kg) in 50% DMSO vehicle, 2 minutes prior to the light pulse. Control groups received either saline or vehicle injections.

Bicuculline blocked the effect of phase delaying light pulses ($p<0.001$), but had no significant effect on phase advancing pulses. The mean phase delay induced by light in the control groups was -0.64±.12 hr., whereas the mean delay under bicuculline blockade was -0.2±.05 hr. The mean phase advances induced by light in control groups and by light+bicuculline were +0.81±.14 hr. and +0.76±.11 hr., respectively. Bicuculline also blocked phase delays induced by saturating light pulses ($p<0.01$; total fluence = 5.5 x 10^{15} photons·cm^{-2}·sr^{-1}).

Preliminary results show that diazepam (25 mg/kg), which may potentiate the effect of GABA through a GABA-benzodiazepine receptor complex, causes phase delays when given without a light pulse at CT 18, a time point at which light would normally cause a large phase advance ($x=-0.3$ hr.;$p<0.05$).

These results suggest that a GABAergic mechanism may be involved in mediating light input to the clock at phase delaying, but not phase advancing time points and that different neurochemical pathways may be involved in mediating phase advances and delays.
(Supported by NIH HD13162 to MM and PHS Training Grant GM07257 to MRR)

313.9 EFFECTS OF DIISOPROPYL PHOSPHOROFLUORIDATE ON CIRCADIAN ACTIVITY PATTERNS IN RATS, J. R. Leu and T. G. Raslear*. Physiology and Behavior Branch, Department of Medical Neurosciences, Division of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D.C. 20307.

Adult, male rats, individually housed under a 12-12 hour light/dark cycle (lights on 0600-1800) were monitored for circadian patterns of feeding. Feeding was monitored by recording either lever presses (with food delivered on a continuous reinforcement schedule) or by recording beam closures on a photocell-type automatic feeding monitor (Kissileff, H. Physiology and Behavior, 5: 163-173, 1970) also delivering food on a continuous schedule. BIOSERV standard ration 45 mg precision pellets were used.

Circadian patterns of feeding were monitored in normal, untreated animals as well as in animals following a single administration of the anticholinesterase compound diisopropyl phosphorofluoridate (DFP). The effects of various pretreatments, including atropine sulfate and 2-PAM chloride, given alone or in conjunction with DFP, were also noted.

In general, the circadian pattern of feeding was altered in DFP treated animals so that a greater proportion of the day's feeding was during the light portion of the light/dark cycle. No significant differences were noted in the total amount of food consumed for the entire day. Preliminary results indicate that atropine and 2-PAM pretreatments were ineffective in attenuating the disruption in feeding patterns of DFP treated animals. These results do not indicate any moderation of feeding cycles in animals receiving the pretreatments alone.

313.10 PROPYLTHIOURACIL CAUSES PHASE DELAYS AND PERIOD LENGTHENING IN ENTRAINED OR FREERUNNING MALE HAMSTERS. L.P. Morin and M.L. Gavin. Dept. Psychiatry, SUNY, Stony Brook, NY 11794.

A thyroid blocking agent, thiourea, combined with thyroidectomy substantially lengthens the circadian period of female hamsters (Beazley and Nelson, 1982). We report that propylthiouracil (PTU), also a thyroid blocker, produces similar effects in intact male hamsters.

PTU was fed to hamsters at a concentration of 6% or 1.2% of the control, powdered diet. The diet sequence was control-PTU-control with diet changes occurring at 3-4 wk intervals. Each animal had access to a running wheel and the phase angle difference (PSI) or circadian period (TAU) was measured during each condition for each animal. 1) Blind animals were fed the control-0.6% PTU-control diet sequence; 2) Similar to Expt. 1, but animals were also pinealectomized or sham operated. After returning to baseline following the diet sequence with 0.6% PTU, the animals were a given 1.2% PTU then returned to the control diet; 3) Sighted animals entrained to LD 14:10 received the control-0.6% PTU-control diet sequence, transferred to LD 6:18 and, after the PSIs stabilized, given the sequence again; 4) Sighted animals in dim LL received the control-0.6% PTU-control diet sequence.

Treeatment with 0.6% PTU lengthened TAU in all animals. Mean change in Expts. 1, 2 and 4 was about 0.2 hr, regardless of lighting or surgical condition. After removal of PTU, average TAU returned to the pre-PTU baseline. The 1.2% PTU did not lengthen TAU beyond that found with 0.6% PTU.

In LD 14:10, no changes in PSI were found associated with the PTU diet. In contrast, PTU given to animals in LD 6:18 induced a mean 2.9 hr phase delay after 2-4 wks of PTU. When PTU was removed, mean PSI advanced 1.4 hr. Unlike the results with TAU, the PTU effects on PSI were not evident in all animals.

The pattern of results is consistent with the idea that the thyroid neuroendocrine axis participates in the regulation of TAU. The level at which this occurs is still obscure.

Supported by NICHD grant HD16231.

313.11 TRANSIENT CESSATION OF PERSISTENT ESTROUS RESPONSE TO CONTINUOUS ILLUMINATION BY LOCUS COERULEUS DAMAGE. L.P. Solano-Flores‡ H.U. Aguilar-Baturoni, O. Donatti-Albarrán‡ C. Santos-Toledo*and R. Guevara-Aguilar. Depto. Fisiología, Fac. Medicina, U.N.A.M. Apdo. Postal 70250, 04510-México, D.F.

We have recently shown that electrolytic damage of the locus coeruleus (LC) results in a transient cessation of estrous cyclicity of the female rat, this fact suggested a modulatory participation of the LC in the normal estrous cyclicity activity. Also, it is known that vaginal smears from rats exposed to continuous bright illumination show an estrous aspect persistently. This work was done with the aim to know whether the LC electrolytic damage disturbs the persistent estrous induced by continuous bright illumination. Vaginal smears were sampled daily during 111 days from mature and virgin Wistar rats. The samples were stained and analysed at 400x magnification. The rats were exposed to continuous bright illumination from the 28 to the 81 day. In the middle of this period the left LC was damaged applying 1.5 mA DC during 20 seconds through a bipolar parallel electrode. As soon as the rats were exposed to continuous illumination the proportion of days in which the vaginal smears showed an estrous aspect was greatly enhanced. Inmediately after the LC damage, the vaginal smears showed a diestrous aspect, this situation was maintained during some days before the smears showed an estrous aspect again. Soon after the normal light-dark illumination was reinstalled, the rats presented estrous cyclicity. In the non-operated and in the operated control animals, a persistent estrous was induced by constant illumination and after the normal light-dark illumination was reinstalled, the estrous cyclicity was again seen. The present data suggest a possible participation of the LC in the activity of those brain regions which mediate the persistent estrous response to continuous illumination.

313.12 DIFFERENTIAL EFFECTS OF SCHEDULED FEEDING ON MELATONIN, N-ACETYL-SEROTONIN AND CORTICOSTERONE RHYTHMS IN RAT SERUM. A.K. Ho‡ T.G. Burns‡ L. J. Grota and G.M. Brown. Department of Neurosciences, McMaster University, Hamilton, Ontario, Canada and Department of Psychiatry, University of Rochester, New York.

Environmental lighting and restricted feeding are known to be capable of entraining biological rhythms. Lighting and pineal N-acetyltransferase (NATase) activity are coupled and therefore both serum and pineal melatonin levels are synchronized with lighting. Serum corticosterone, however, can be entrained by restricted food availability. N-acetylserotonin (NAS) is thought to be synthesized exclusively in the pineal by action of NATase on serotonin, and its serum levels synchronized with environmental lighting; as is serum melatonin. Hence, serum levels of these indoles were believed to be tightly coupled, but recent evidence has demonstrated that the rhythms of serum NAS and melatonin can be dissociated under certain lighting conditions. This suggests that there are independent mechanisms of regulation of their serum levels, and we investigated the possibility that serum NAS may be synchronized with the other known entrainer, feeding.

Three groups of male rats were maintained for 4 weeks under similar light-dark cycles; LD 14:10. Group A had access to food for a 3-hour period at the beginning of the light period, while Group B had access during the beginning of the dark period. Group C was identical to Group B, except that they were food deprived on the experimental day. Serum melatonin, NAS, and corticosterone were measured by radioimmunoassay. Serum NAS in both groups A and B demonstrated a trough about 8 hours prior to feeding and a crest during the time of feeding. In the case of serum corticosterone rhythm, peaks were observed in both groups A and B prior to the feeding time. However in the case of group A, an extra peak was present at the beginning of darkness. This suggests that scheduled feeding for four weeks is not adequate in totally shifting the corticosterone rhythm. As expected, serum melatonin demonstrated peak levels about 8 hours into the dark in all groups i.e. showed no relationship to the time of feeding. Of particular interest was the finding that in group C, the food deprived animals, the serum levels of NAS were identical to those of group B, indicating that after 4 weeks of entraining the rhythm of circulating NAS, though synchronized with the time of feeding, can occur in the absence of feeding. These findings strongly suggest that the rhythms of circulating NAS and corticosterone are synchronized with the time of feeding, while that of serum melatonin is dependent on environmental lighting conditions. Furthermore, serum NAS and melatonin rhythms may be synchronized by different types of environmental entrainers.

313.13 DEVELOPMENT OF ACTIVITY RHYTHMS IN RATS REARED IN ISOLATION FROM THE DAM. V.Anderson* and G.K.Smith, Dep't of Psychology, McMaster University, Hamilton,Ontario, Canada L8S 4K1.

Prenatal and postnatal maternal factors have been shown to influence the phase of circadian locomotor activity rhythms in rodents(Davis & Gorski, Neurosci. Abstr., 1982) but there is little information on the development of activity rhythms in the absence of maternal stimuli. We have continuously recorded locomotor activity in rat pups reared from day 3 to day 18 of postnatal age (day of birth=day 0) in isolation and fed an artificial diet through an indwelling gastric catheter (Hall, Science, 190:1313, 1975).

Autocorrelation analysis revealed that seven(n=12) of the animals reared in isolation on a 12:12 LD cycle (ARLD group) exhibited circadian activity rhythms with greater activity during the dark period($\tau \approx 24$hrs). The mean age at onset was 9 days and the rhythms persisted for approximately 3 days. In contrast, all of the pups in a mother-reared control group(MRLD,n=7) showed circadian rhythms($\tau \approx 24$hrs) starting at 9 days of age and lasting until weaning at 18 days. Only two pups reared in isolation under continuous light(ARLL, n=9) showed circadian rhythms(τ= 23 and 28.5hrs)lasting two days. Although day-night differences existed in the ARLD group the mean proportion of activity at night never rose above 56.5% from 11 to 18 days of age. In the MRLD group night activity was consistently 60% or more after 11 days of age. Preliminary analysis of an isolated group reared on a reverse light cycle indicates that the day-night differences are probably due to the LD cycle and not some other cyclic environmental factor.

The persistence of the circadian rhythms and day-night differences in the mother-reared rats suggests that the dam may have been synchronizing the offspring's activity rhythms. In the absence of the dam (ARLD group) transient circadian rhythms may appear. When light-dark and maternal cues are missing(ARLL group) circadian rhythms are even less frequent and the period is more variable. These data also demonstrate that circadian locomotor activity rhythms in rats may appear at ages younger than those previously reported in the literature (eg., Teicher & Flaum, Dev. Psychobiol., 12:441, 1979).

313.14 **FIVE HOURS OF LIGHT PER CIRCADIAN CYCLE, ADMINISTERED NONPARAMETRICALLY VIA FEEDBACK CIRCUITS, CAUSES ANOVULATION AND LONG FREERUNNING PERIODS IN THE FEMALE RAT.** J.S. Ferraro* and C.E. McCormack* (Spon: P.C. Tang). Dept. of Physiol. Biophys., The Chicago Med. Sch., N. Chicago IL. 60064.

Exposure of female rats to continuous light (LL) causes cyclic ovulation to cease and the circadian rhythm of locomotor activity to freerun with a long period (τ). It is not known whether these effects of LL are due to the parametric effects of light (i.e. the rat being exposed to too many hours of light per circadian cycle) or to the nonparametric effects of light (i.e. the rat being exposed to light during sensitive portions of it's circadian cycle). Since the phase of the locomotor activity rhythm in rats can only be shifted by light which falls on the active portion of the rhythm, it seems possible that only a small portion of the light is responsible for the long freerunning τ in LL. The purpose of this experiment was to expose solely the sensitive portions of the rat's circadian cycle to light. Making the assumption that the light-sensitive portions of the circadian cycle would parallel locomotor activity, we designed feedback circuits which turned on a 100 lux light source for a duration of 2 min. whenever the rat rotated the wheel at a rate of 8 revolutions/min. [Although held constant in this experiment, the rate-criterion and the duration of "lights-on" are adjustable.] Prior to exposure to feedback lighting (LD_{FB}), mature female Charles River rats displayed regular estrous cycles while on activity wheels. Although exposure to LD_{FB} forced the rats to run in light, they nevertheless ran their normal 5h per circadian cycle spread out over the usual 12-14h interval. Circadian onsets of activity were readily discernable, and moreover were clearly freerunning. The mean τ in LD_{FB} for 12 rats was 25.7h. When 2 of these rats were placed in an identical intensity (100 lux) of LL, their τ's changed only slightly (25.7LD_{FB} $\rightarrow$ 25.3LL; 25.5LD_{FB} $\rightarrow$ 25.4LL). Whether in LD_{FB} or LL, regular cyclic ovulation usually ceased within 8 days (mean of 5 days). Our criterion for loss of ovulation was 3 consecutive days of complete vaginal cornification, established by twice-a-day vaginal smearing. Two rats exposed to LD_{FB} (1 lux) and one to LD_{FB} (0.1 lux) had τ's of 25.1, 25.0, and 24.4h respectively, and displayed regular estrous cycles for longer periods of time. These preliminary results indicate that the well known effects of LL on τ, and on the loss of cyclic ovulation result from the nonparametric effects of light. Moreover, these effects appear to be directly related to light intensity as described by Aschoff's circadian rule. Supported by NIH-HD 13131.

313.15 INTERACTIONS AMONG THE DAILY PATTERNS OF FEEDING, WHEEL-RUNNING, AND LOCOMOTOR ACTIVITY IN SYRIAN HAMSTERS. J.E. Ottenweller*, W.N. Tapp*, G.A. Curtis*, and B.H. Natelson. (SPON: G.A. Condouris). Dept. of Neurosciences, UMDNJ-New Jersey Med. School, East Orange, NJ 07018.

The present study examined the daily patterns of feeding, wheel-running, and locomotor activity in Syrian hamsters (Mesocritus auratus) to elucidate possible interactions among these behaviors. Young male hamsters (2 months) were placed on a 12h:12h LD cycle (onset of light: 0730h) with food and water available ad libitum. After 3 weeks, hamsters were transferred to cages in which their activity was monitored. Locomotor activity was assessed by counting cage crossings using tilt cages, feeding was monitored by counting entries into feeders, and wheel-running was measured by counting turns of hamster running wheels. Counts were collected in 10 min. bins for several weeks by a computer, stored, and then analyzed by power spectrum analysis to determine what periodicities were present in these behaviors. No circadian rhythms were detected in feeding records, but 80-90 min. and 40-50 min. ultradian rhythms were found throughout the day. As expected, circadian rhythms were present in wheel-running activity with almost all running confined to times of day when lights were off. Cage crossing records appeared to combine aspects of both feeding and running wheel records, in that greater activity occurred during times of day when lights were off and yet periodic episodes of cage crossing activity occurred throughout the day. Thus, cage crossing records were characterized by both circadian periodicities similar to running wheel records and ultradian periodicities similar to feeding records. Inasmuch as cage crossing records show much more diurnal activity than running wheel records, these data indicate that running wheels measure only a fraction of total locomotor activity. When locomotor activity (cage crossings) and wheel running were monitored concurrently, locomotor activity records were more circadian than when they were monitored in the absence of running wheels. In contrast, the presence of running wheels did not alter feeding records. These data suggest that wheel running may function to enhance the circadian organization of locomotor activity but not feeding. (Supported by VA Medical Research Funds).

313.16 **NORMAL HUMAN RHYTHMS OF PLASMA AND URINARY 3-METHOXY-4-HYDROXYPHENYLGLYCOL (MHPG) AND ORAL TEMPERATURE.** E. DeMet, H. Gwirtsman*, A. Halaris, Dept. of Psychiatry, UCLA Schl. of Med. and VA Med. Cntr. West Los Angeles, Los Angeles, CA 90073.

A number of studies have suggested that changes in plasma and urinary MHPG levels may reflect changes in central noradrenergic activity. More recently, several reports have demonstrated that diurnal rhythms of plasma MHPG, urinary MHPG and body temperature may be altered in psychiatric disorders. The present study examined the variance and temporal relationships of these rhythms in normal human subjects.

Plasma MHPG and oral temperature were measured at 3 hour intervals for at least 24 hrs. Urinary MHPG and creatinine levels were determined on 2 consecutive 12 hr. samplings during the same period (9AM;9PM). Individual plasma MHPG profiles were best fit to a cosine function by nonlinear regression and Gauss-Newton convergence. Unlike linear regression models, the present methods permitted an estimation of the diurnal period as well as the amplitude, baseline, and phase of the individual rhythms. Plasma MHPG values (ng/ml) and standard errors were: 3.53 $\pm$ 0.41, baseline; and 1.43 $\pm$ 0.14, amplitude. The diurnal acrophase occurred at 15.00 $\pm$ 0.77 hrs. and the estimated period was 22.28 $\pm$ 1.39 hrs.

To permit a direct comparison between plasma and urinary MHPG values, the best fit plasma curves were integrated over 12 hr. intervals. To estimate the phase relationship between plasma and urine, the integration intervals were iteratively evaluated starting from time 1 to 12 hrs. The correlation between plasma and urinary MHPG levels was evaluated across subjects for each iteration. The resulting set of 12 correlation coefficients formed a sinusoidal curve which was best fit to a cosine function. The phase delay of urinary MHPG excretion relative to plasma levels was estimated from the position of the peak correlation to be 6.73 hrs. The linear correlation between phase adjusted plasma and urinary MHPG was r = 0.80.

The phase relationship between plasma MHPG and oral temperature was estimated by best fit of each data set to a cubic polynomial. No significant difference was found between the acrophase for plasma MHPG (14.88 $\pm$ 0.81) and oral temperature (15.83 $\pm$ 2.45).

The results indicate that plasma MHPG rhythms were synchronized with oral temperature rhythms in the normal subjects studied. Urinary excretion of MHPG lagged behind the plasma MHPG rhythm by nearly 7 hours. When the plasma levels were adjusted for this phase difference, a linear relationship was found between plasma levels and urinary excretion.

Support by Grant MH35824 NIMH and by the Veterans Administration.

313.17 ALGORITHMS FOR THE PRODUCTION OF TIMING SEQUENCES IN HUMANS. D. Hary* and G. P. Moore Dept. of Biomedical Engineering, University of Southern California, Los Angeles, Calif., 90089-1451.

In a study in which we are attempting to assess the limits of human performance in temporal precision we have compared a control group of subjects with a group of highly trained musicians

Subjects were instructed to tap a morse key in synchrony with an audible metronome whose period ranged from 250 to 2500 msec. In a continuation task subjects were instructed to maintain the same interval of tapping without the metronome. Auto- and cross-correlation functions of the key tapping and metronome event sequences were calculated, as well as the auto- and cross-serial correlograms of intervals and errors. Also tested was the stationarity of the continuation performance Although the musicians in our study all performed at significantly higher levels of temporal precision, the data analyses showed that they frequently adopted different, albeit unconscious, strategies for timing that minimized a particular type of error. These strategies could therefore be altered structurally by the specific instructions given to the subject. In some paradigms, performances of very high precision could be simulated by relatively simple algorithmic models, and these allow us to estimate an upper bound for the noise in the internal timing processes of humans

(Supported by a grant from the Ford Motor Company to the University of Southern California)

HORMONAL CONTROL OF BEHAVIOR I

314.1 HYPERPROLACTINEMIA AFFECTS COPULATORY BEHAVIOR WITHOUT ALTERING DOPAMINERGIC FUNCTION OUTSIDE THE MEDIAN EMINENCE P.C. Doherty, S. Lane*, K. Pfeil*, W.W. Morgan, A. Bartke* and M.S. Smith*. Depts. of Anat and Ob.-Gyn., Univ. of Texas Hlth. Sci. Ctr San Antonio, TX 78284, and Dept. of Physiol , Univ. of Pittsburgh Sch of Med., Pittsburgh, PA 15261.

Recent evidence suggests that prolactin (PRL) may affect dopaminergic function in brain areas other than the median eminence Since central dopamine is believed to influence the expression of male sexual activity we have investigated the possibility that the suppression of copulatory behavior in hyperprolactinemic male rats may be associated with chronic changes in central dopaminergic function. Animals which had received 4 ectopic pituitary grafts under the kidney capsules showed increased serum PRL levels (549.8 $\pm$ 37.4 vs 68.5 $\pm$ 8.4 ng/ml) and suppressed copulatory behavior in comparison to sham-operated controls. The decline in DA concentrations in terminal fields of the nigrostriatal, mesolimbic, incerto-hypothalamic and tuberoinfundibular DA pathways 2 hr after an i.p. injection of 250 mg/kg of α-methyl-p tyrosine (αMPT) were measured in these animals 19 days after pituitary transplantation

Significant effects of hyperprolactinemia were observed only in the median eminence, where pituitary-grafted animals showed both a significant reduction in steady state DA concentrations (49.9$\frac{1}{4}$ $\pm$ 4 25 vs 73.75 $\pm$ 4 21 ng/mg-protein; $p<0.001$) and a significant reduction in the fractional amount of DA remaining in the median eminence of α-MPT-treated rats (0.21 $\pm$ 0.02 vs 0 58 $\pm$ 0.07; $p<0.001$) when expressed per mean DA concentration of non α-MPT treated controls. To further assess the affects of hyperprolactinemia on central dopaminergic function, the accumulation of DOPA after administration of 100 mg/kg NSD-1015 was examined in animals injected twice daily with 400 µg ovine PRL. PRL-treated animals showed significant suppression of sexual activity in comparison to vehicle treated controls However, DOPA accumulation was significantly increased only in the median eminence of the PRL-treated group (94 39 $\pm$ 6.86 vs 36.33 $\pm$ 4.08 ng DOPA/mg-protein; $p<0.001$) and not in other brain areas examined. In addition, no significant differences were observed between pituitary-grafted and sham-operated animals in locomotor response to the open-field or in stereotyped behavior in response to 0 2, 0.5 and 1.0 mg/kg of apomorphine.

No differences were found in the characteristics of ^{3}H-spiroperidol binding to striatal homogenates of PRL and vehicle treated animals. Since tuberoinfundibular DA neurons are not believed to influence behavioral expression, these results suggest a lack of involvement of changes in central dopaminergic function in the suppression of copulatory behavior in hyperprolactinemic male rats.

Supported by NIH grants HD 12671 and DA 00755.

314.2 TEMPORAL RELATIONSHIP BETWEEN ONTOGENY OF SEX HORMONE RECEPTORS AND SEXUAL DIFFERENTIATION IN HAMSTER BRAIN. C. C. Vito and J. F. DeBold. Depts. of Neuroscience Children's Hosp. Med. Ctr., Boston, MA 02115 and Psychology, Tufts Univ., Medford, MA 02155.

In our previous studies of mice, rats and ferrets, we characterized the sex hormone receptors in brain during a sensitive period of behavioral differentiation. In each species, there appears to be a correlation between the ontogeny of receptors and the time course of each sensitive period. To pursue these observations further, we have begun a similar analysis of receptors in developing hamster brain.

Using ^{3}H-dihydrotestosterone (DHT), ^{3}H-estradiol and DNA-cellulose affinity chromatography, we have characterized the androgen- and estrogen-binding activities in the hypothalamus-preoptic area (HPOA) of male and female hamsters ranging in age from embryonic-day 15 (E15) to postnatal-day 21 (P21) (day of mating = E0; birth = E16, P0); the activities detected in cytosol extracts of developing HPOA exhibit chromatographic behaviors which are similar to the androgen and estrogen receptors in adult HPOA.

We have also examined the affinities of these hormone-binding activities. The ^{3}H-DHT-binding activity in the HPOA of intact males and females at P10 is 88.5 $\pm$ 1.8% (SEM, n = 4) saturated at a concentration of 10 nM; at P21 is 97.0 $\pm$ 4.7% (n = 4) saturated; at P60 is 108 $\pm$ 0.8% (n = 4) saturated. The ^{3}H-estradiol-binding activity at P10 is 76.9 $\pm$ 3.5% (n = 8) saturated at a concentration of 5 nM and 90.5 $\pm$ 3.4% (n = 4) saturated at 10 nM; at P60 is 100.3 $\pm$ 2.6% (n = 4) saturated at 10 nM. We have also begun to investigate the ontogeny of androgen and estrogen receptors in hamster HPOA. The concentration of putative androgen receptors in the HPOA of intact males and females at E15 is 0.33 fmoles/mg protein (n = 2); at P0 is 0.34 $\pm$ 0.03 (n = 3); at P2-3 is 0.58 (n = 2); at P4-5 is 1.26 (n = 2); at P10 is 2.09 $\pm$ 0.07 (n = 6); at P14-21 is 2.12 $\pm$ 0.23 (n = 13). This gradual increase in androgen receptor concentrations in developing hamster HPOA is similar to that which occurs in mice and rats. In contrast, estrogen receptor concentrations appear to undergo a more rapid increase, especially during the early postnatal period. The concentration of putative estrogen receptors in HPOA at E15 is 0.80 fmoles/mg protein (n = 2); at P0 is 0.86 (n = 2); at P2-3 is 1.41 (n = 2); at P4-5 is 2.27 $\pm$ 0.18 (n = 3); at P10 is 2.37 $\pm$ 0.17 (n = 9); at P16-21 is 2.45 (n = 2). Thus, the early postnatal period (P0-P5) in hamster HPOA, which is a sensitive period of brain sexual differentiation in hamsters, is characterized by increases in the concentration of both receptors, with postnatal estrogen receptor levels rising rapidly and reaching an early plateau. A similar developmental pattern is observed in the HPOA of rats and mice and is coincident with their sensitive period which begins prenatally.

[Supported by NIH Grant #HD16118 (CCV) and a Biomedical Research Grant (JFD)]

314.3 COPULATORY DEFICITS FOLLOWING OLFACTORY BULBECTOMY: POSSIBLE ANDROGEN RECEPTOR INVOLVEMENT. A.R.Lumia, A. Zebrowski and M.Y. McGinnis (SPON:T.L.Schwartz). Dept. Anatomy, Mount Sinai School of Medicine, New York,N.Y. 10029 and Dept. Psychology, Skidmore College, Saratoga Springs,N.Y. 12866.

Bilateral olfactory bulbectomy (BOB) virtually eliminates effective copulatory activity in male rats. This behavioral deficit is not due to impaired androgen secretion. The olfactory bulbs have neural connections with ventral hypothalamus,preoptic area, medial amygdala and septum which are major neural sites of intracellular androgen receptors in brain. Thus we hypothesized that copulatory deficits following BOB in males could result from loss of neural substrates for androgen receptor binding. Male Long-Evans rats were castrated and implanted sc with 2 x 10mm testosterone-filled Silastic capsules, and pretested for copulatory behavior. Rats copulating through two ejaculatory series received either bilateral aspiration of the olfactory bulbs or Sham surgery. Post-operative tests were conducted 1-2,3-5,6-7 and 8-9 days after surgery to determine the onset of the copulatory deficits. Following behavior tests,rats were sacrificed for assessment of cell nuclear androgen receptor occupation in hypothalamus, preoptic area, amygdala and septum using an exchange assay (McGinnis et al.,Brain Res.1983). Relative to Shams, a significantly lower proportion of BOB rats copulated to two ejaculations on all test days, and significantly fewer BOB rats displayed intromission patterns on all but days 8-9. Cell nuclear androgen receptor levels in the hypothalamus of BOB males were significantly lower than in Shams. Androgen receptor levels were also lower in the amygdala of BOB rats, but this was not statistically significant. There were no differences in androgen receptor levels in preoptic area or septum between BOB and Sham rats. Our results indicate that the copulatory deficits resulting from BOB are associated with decreased androgen receptor binding in the hypothalamus, suggesting that loss of neural substrate for androgen receptor binding may, at least in part, acccount for the decrements in copulatory behavior found in BOB males. However, the rapid appearance (1-2 days post-op) of the copulatory deficit suggests that other neural factors may be involved.

Supported by grants from The Mount Sinai Medical Center, Skidmore College and the Andrew Mellon Foundation.

314.4 CORRELATION OF MUSCARINIC RECEPTOR INDUCTION IN THE VENTROMEDIAL NUCLEUS WITH THE ACTIVATION OF FEMININE SEXUAL BEHAVIOR BY ESTRADIOL. B.S. McEwen, L. Snyder*, and T.C. Rainbow. The Rockefeller University, New York, NY 10021; and Dept. of Pharm., University of Pennsylvania Medical School, Philadelphia, PA 19104.

The administration of the steroid hormone, estradiol (E_2), to ovariectomized rats will increase by 20-40% the levels of muscarinic cholinergic receptors in the hypothalamic area (Rainbow et al., Brain Res. 1980; Dohanich et al., Brain Res. 1982; Olsen et al., Soc. Neurosci. Abstr., 1982). By microanatomical dissection methods, it has been shown that this increase is confined to hypothalamic subregions that possess high levels of intracellular E_2 receptors, among which is the ventromedial nucleus (VMN), the principal target site for the activation of feminine sexual behavior by estradiol. We have extended our previous findings and report that the increase in VMN muscarinic receptors satisfies many of the criteria for an important molecular mechanism by which E_2 could activate sexual behavior. The increase in VMN muscarinic receptors as determined by the binding of (^{3}H) QNB to microdissected homogenates occurred by 18-24 hr after exposure to E_2 from a subcutaneously-implanted Silastic capsule, the earliest time at which sexual behavior is facilitated. The increase does not occur in the VMN of male rats, which show little activation of feminine sexual behavior after E_2 plus progesterone exposure. Muscarinic receptors in the VMN of female rats are also induced at 24 hr by a 6 hr exposure to E_2, the minimum length of time that is sufficient for E_2 to activate sexual behavior. A 4 hr exposure to Silastic E_2 is insufficient to activate both sexual behavior and induce muscarinic receptors. Administration of the protein synthesis inhibitor, anisomycin, during the 6 hr E_2 exposure will block both the increase in muscarinic receptor levels and the activation of feminine sexual behavior. These results are similar to results obtained for progestin receptor induction by E_2 and suggest that the muscarinic cholinergic receptor may be one of the proteins induced by E_2 in the VMN to activate feminine sexual behavior. We have also used quantitative autoradiography to further characterize the effects of E_2 on VMN muscarinic receptors. Using saturation analysis, it was found that E_2 increases the total number of muscarinic sites in the VMN without affecting the affinity of the binding. The relative proportion of carbachol-displacable sites is unchanged in the VMN after E_2 treatment, indicating that the effects of E_2 occur equally on low and high-affinity muscarinic receptors. These results are consistent with E_2 acting directly on VMN neurons to induce muscarinic receptors. (Supported by NS07080, NS20006, NS19597, a Sloan fellowship to TCR and an institutional grant RF81062 from the Rockefeller Foundation.)

314.5 AN EXCHANGE ASSAY FOR THE MEASUREMENT OF NUCLEAR ESTROGEN RECEPTORS (ERn) IN MICRODISSECTED HYPOTHALAMIC NUCLEI. J. Kranzler, E. Jones*, H. Sakamoto*, N. MacLusky* and F. Naftolin*. Depts. of Pharmacology and Ob/Gyn, Yale Univ. Sch. of Med., New Haven, CT 06510.

Palkovits' punch-out technique (Brain Res. 59:449, 1973) has facilitated the study of hormone receptors in the brain with a high degree of anatomic resolution. Using this procedure, Rainbow, et al., (J. Neurosci. 2:1439, 1982) have mapped the distribution of cytoplasmic estrogen receptors (ERc) in the rat brain, and have demonstrated a sex difference in the concentration of ERc in the medial preoptic nucleus (Nature 300:648, 1982). However, it has not so far been possible to measure ERn in individual hypothalamic nuclei, under physiologic conditions of estrogen (E) receptor occupation. We have modified the nuclear exchange assay of Roy and McEwen (Steroids 30:657, 1977) to quantitate ERn in punch-out samples. Our technique uses cellulose (Bio-Rad Cellex 410) as a carrier for the isolation of the nuclear pellet.

Female Sprague-Dawley rats (55-70 d) were injected i.v. with a saturating dose (3.6 µg/kg) of estradiol (E2). One hour later, they were perfused with a 10% DMSO solution, and the brains removed, mounted, and frozen. Sections (300 µm) were cut and 6 high E-binding regions (periventricular preoptic area, PVPOA; medial preoptic area, MPOA; periventricular anterior hypothalamus, PVAH; medial amygdala, AM; arcuate-median eminence, AR-ME; and, ventromedial nucleus, VMN) microdissected. Individual nuclei from 5 animals were pooled and homogenized in isotonic phosphate-sucrose buffer (NII). The homogenate was mixed with 2 volumes of 2.4 M sucrose solution (NIII) containing 0.25% Triton X-100 and layered over 2.1 M sucrose. A small amount of cellulose suspension was added at the top of the tube, and the three layers spun at 20,000 x g for 30 min. The final nuclear pellet contained only cellulose fibers and cell nuclei, as determined by light microscopy. ERn were measured by exchange assay, as previously described (Clark, et al., J. Endocr. 93:339, 1982).

Mean ERn concentrations (fmoles/mg DNA) in each nucleus studied by exchange assay were as follows: PVPOA = 416; POA = 378; PVAH = 134; AM = 153; AR-ME = 170; VMN = 65. These ERn levels are similar to the specific E2 binding observed in the same nuclei after i.v. injection of [3H] E2, followed by extraction and counting of the nuclear pellet. In females, the relative ERn concentrations parallel the distribution of ERc in the same nuclei. Preliminary results suggest, however, that there is no sex difference in the MPOA at the ERn level after E2 administration. (Supported by HD13587).

314.6 ESTROGEN AND MONOAMINES IN BRAIN NUCLEI: DOSE EFFECTS AND TIME COURSE OF ESTROGEN ACTION. K.J. Renner* and V.N. Luine (SPON: S.Schwartz-Giblin). Rockefeller University, New York, NY 10021.

Monoamines have been implicated in the neural control of sexual receptivity and gonadotropin secretion using experimental approaches such as systemic and central applications of monoamine agonists and antagonists and direct neurotransmitter infusions into the brain. Further evidence for gonadal steroid-monoamine interactions are suggested by fluctuations in catecholamine levels in specific brain nuclei during the estrous cycle (Crowley et al., Brain Res. 147:315, 1978). In the present study, the effect of estrogen on monoamine levels in hypothalamic, preoptic and limbic nuclei of ovariectomized rats was examined. Norepinephrine (NE), dopamine (DA), serotonin (5HT), and the 5HT metabolite 5-hydroxyindole acetic acid (5HIAA) were simultaneously measured in individual animals using high performance liquid chromatography with electrochemical detection. Monoamine levels were determined in the following brain nuclei: ventromedial hypothalamus (VMN), dorsomedial hypothalamus, anterior hypothalamus, suprachiasmatic, arcuate-median eminence, medial preoptic (POA), lateral septum (LS), medial and cortical amygdala, diagonal band of Broca (DBB), and periventricular regions. Estrogen effects were assessed as a function of dose (1,5,10 and 50µg) after 24 hrs or time after a 5µg dose (1.5, 6,12,24 and 45 hrs). Estrogen induced small, dose-dependent increases in NE content in the DBB (18-84%) and 5HIAA content in the VMN (14-48%). Increased NE content in the POA did not appear to be dependent on dose of estrogen given. Increases in VMN 5HIAA were rapid, 52% at 6 hrs, while changes in NE content in the DBB and POA did not occur until 12 hrs with maximal increases present at 24 hrs. In addition, increased NE content was found in the LS (31%) and periventricular region (39%) 45 hrs after estrogen administration. Dopamine content in the arcuate median eminence was decreased by 32% at 45 hrs. Estrogen did not affect monoamine content in any other nuclei sampled.

These results suggest that estrogen exerts selective dose and time dependent changes in some neurotransmitters within specific brain nuclei when administered in doses sufficient to activate sexual receptivity. The varied nature of the changes may reflect complex hormonal-neural interactions and suggest that estrogen may act both through genomic and non-genomic mechanisms to alter monoaminergic neurotransmission.

(Supported by USPHS Grant HD12011 and USPHS Postdoctoral Grant HD06368).

314.7 PROTEIN SYNTHESIS AND THE REGULATION OF SEXUAL BEHAVIOR AND NEURONAL ULTRASTRUCTURE BY ESTROGEN IN THE FEMALE RAT. R. L. Meisel and D. W. Pfaff. The Rockefeller University, New York, NY 10021.

It is commonly accepted that many steroid hormone effects on target tissues are mediated via regulation of the genome. We are investigating the role of neuronal protein synthesis in the hormonal activation of female sexual behavior (lordosis) using intracranial implants of anisomycin (Rainbow et al., Brain Res. 233: 417), a protein synthesis inhibitor of low toxicity. In the first experiment, ovariectomized female rats received bilateral cannula implants in the medial preoptic region (POA), septal region (SEPT), ventromedial hypothalamus (VM), or midbrain central gray (CG). All females were injected sc with 2.5μg estradiol benzoate (EB) followed by 500μg progesterone (P) 46 hr later, with intracranial anisomycin applied at the time of EB injection. Females with anisomycin implants in the VM had lower levels of lordosis than females with implants in the POA, SEPT, or CG. In a second experiment, we replicated the finding that anisomycin could attenuate lordotic responsivity when placed in the VM of hormone-primed female rats. In addition, we found that POA implants of anisomycin could facilitate lordosis in ovariectomized females given a low dose of EB (1.25μg) plus 500μg P. In a third experiment, we assessed the effects of anisomycin application to the VM or POA of ovariectomized female rats receiving estradiol implants (E; diluted 1:250 with cholesterol) in the VM and systemic P. Treatment of the VM with anisomycin prior to E in the VM suppressed lordotic responding, whereas anisomycin application to the POA prior to E in the VM had no effect on lordosis. The results of these experiments suggest that reducing protein synthesis in the VM disrupts the action of estrogen on the VM, and that the facilitative action of anisomycin in the POA of female rats requires more estrogen treatment than threshold stimulation of the VM alone.

In a companion series of experiments, we are investigating the effects of estrogen on ultrastructural indices of protein synthesis in the female rat brain. It was shown previously that estrogen treatment increases the percentage of neurons containing stacks of rough endoplasmic reticulum (ER), as well as the number of dense-cored vesicles, in neurons of the ventromedial hypothalamus (Cohen & Pfaff, Cell Tissue Res. 217:451). In the arcuate nucleus, changes in these organelles correlate with the stage of the estrous cycle, with the highest levels occurring during diestrus (King et al., Cell Tissue Res. 153:497). Preliminary indications are that among estrogen-concentrating brain regions (e.g., arcuate nucleus, POA, CG), estradiol increases ER stacking in some regions (e.g., VM), while inhibiting (e.g., arcuate) or having no effect (e.g., CG) on ER in neurons of other regions.

314.8 ANDROGEN AND ESTROGEN RECEPTORS IN ZEBRA FINCH BRAIN. L. I. Siegel, T. O. Fox and M. Konishi. Department of Neuroscience, Children's Hospital and Neuropathology, Harvard Medical School, Boston, MA 02115, and Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Perinatal exposure to gonadal hormones influences sexual differentiation of brain function in mammals and birds. In songbirds such as the zebra finch, singing is a sexually dimorphic behavior which is controlled by a system of interconnected brain nuclei (Nottebohm et al., J. Comp. Neurol., 1976, 165, 257). Sex differences in the volume and cytoarchitecture of song nuclei correlate well with the sexual dimorphism in behavioral capacity for song, and data suggest that androgens and estrogens participate in the development and maintenance of these differences (Gurney, Brain Res., 1982, 231, 153; Gurney and Konishi, Science, 1980, 208, 1380). Although autoradiography indicates that adult zebra finch brain can accumulate radioactivity in regions including song nuclei after injection of ^{3}H-testosterone (Arnold et al., J. Comp. Neurol., 1976, 165, 487), uptake of ^{3}H-estradiol (^{3}H·E) in forebrain song nuclei has not been reported (Arnold, Soc. Neurosci. Abstr., 1979, 5, 437). Thus, we sought to demonstrate the existence of both androgen and estrogen receptors in zebra finch brain.

Analysis by DNA-cellulose chromatography of specific brain regions, including telencephalic areas containing nuclei mediating song, indicates the existence of both androgen- and estrogen-binding activities. Tissues were homogenized at 2° C in buffer consisting of 50 mM Tris-HCl (pH 8.1, 21° C), 1 mM Na_2EDTA, 10 mM NaCl, and 1 mM mercaptoethanol or 1 mM dithiothreitol. Extracts were centrifuged and resultant fractions were incubated with ^{3}H-dihydrotestosterone (^{3}H·DHT) or ^{3}H·E. Radioactively labeled extracts were loaded on DNA-cellulose columns, incubated, and washed. Radioactivity was then eluted with a linear concentration gradient of NaCl, revealing two regions of binding activity. One binds ^{3}H·DHT and elutes between 110 and 160 mM NaCl, as is characteristic of androgen receptors in other species. The second binds ^{3}H·E and elutes between 170 and 210 mM NaCl, as do estrogen receptors in other species. ^{3}H·DHT- and ^{3}H·E-binding are blocked by competition with non-radioactive DHT and E, respectively. We find similar binding activities in newly-hatched chicks (prior to sexual differentiation of telencephalic song nuclei).

In adults of either sex, more ^{3}H·DHT-binding is found than is ^{3}H·E-binding in all regions studied. In general, more saturable ^{3}H·DHT-binding is observed in adult male brain compared to adult female brain.

The demonstration of androgen- and estrogen-receptors in newly-hatched and adult zebra finch brain provides evidence for biochemical mediators through which gonadal hormones might act to effect changes in neuronal tissue, including song nuclei.

(Supported by NIH grant [NICHD] HD-10818 [to TOF], NINCDS traineeship 5-T32-NS07017 [to LIS], and a grant from the Whitehall Foundation [to MK]. Facilities supported by Mental Retardation Research Center Core Grant HD06276.)

314.9 PROGESTERONE PROLONGS THE DURATION OF HEAT IN FEMALE GUINEA PIGS: CORRELATION WITH RETENTION OF NEURAL NUCLEAR PROGESTIN RECEPTORS. Theodore J. Brown* and Jeffrey D. Blaustein (SPON: D. G. Emery). Dept. of Zoology, Iowa State Univ., Ames 50011 and Dept. of Psychology, Univ. of Massachusetts, Amherst 01003.

In female guinea pigs, estradiol and progesterone interact to activate a period of sexual receptivity (heat) of an 8-10 h duration. Previous studies indicate that the retention of nuclear progestin receptors (PRs) in the medial-basal hypothalamus and preoptic area (MBH-POA) following a progesterone injection correlates with the expression of sexual receptivity. We have shown that both estradiol and progesterone regulate the level of nuclear PRs in these tissues. In this study, we test the hypothesis that progesterone influences both heat duration and retention of nuclear PRs in the MBH-POA.

In Experiment 1, the effect of continuous administration of progesterone on heat duration was determined. Ovariectomized (OVX) guinea pigs were implanted with a 5 mm Silastic capsule containing 5% estradiol. Forty h later, animals were implanted with a 0.5, 1.5, 3.0, 4.5, or 6.0 cm capsule containing progesterone and were tested hourly for lordosis. Heat duration was shorter in animals receiving 4.5 or 6.0 cm capsules than in animals receiving 3.0 cm capsules; however, heat termination occurred at about the same time regardless of capsule length. In Experiment 2, the effect of a supplemental injection of progesterone on heat duration was tested. OVX guinea pigs were injected with 4 μg estradiol benzoate followed 48 h later with 50 μg progesterone. Animals were tested hourly for lordosis. Eight h after the first progesterone injection, animals received either 500 μg progesterone or vehicle. Supplemental progesterone increased heat duration and delayed heat termination by more than two h. In fact, by 6 h after treatment, most of the supplemental progesterone-treated animals were still sexually receptive, whereas control animals were no longer in heat. In Experiment 3, MBH-POA cytosol and nuclear PRs were measured in OVX guinea pigs treated as in Experiment 2 to determine if the progesterone-induced prolonged heat duration correlated with a prolonged retention of nuclear PRs. Six h after treatment, PR levels were still elevated in the supplemental progesterone-treated animals, while levels in control animals had returned to baseline. These results are consistent with the behavioral results of Experiment 2.

These experiments confirm that under some conditions, supplemental progesterone treatment can prolong heat duration. They also further the correlation between the occurrence of sexual receptivity and the retention of nuclear progestin receptors in the MBH-POA.

(Supported by NSF grant BNS 13050)

314.10 PROGESTERONE DECREASES THE CONCENTRATION OF HYPOTHALAMIC AND ANTERIOR PITUITARY ESTROGEN RECEPTORS IN CHRONICALLY ESTROGEN-TREATED OVARIECTOMIZED RATS. Jeffrey D. Blaustein and Theodore J. Brown*. Dept. of Zoology, Iowa State University, Ames, IA 50011 and Dept. of Psychology, University of Mass., Amherst, MA 01003.

Progesterone inhibits the effect of chronic estradiol treatment on a variety of behavioral and neuroendocrine end-points. In a study of cellular mechanisms of progesterone's antiestrogenic action, we investigated the influence of progesterone on the concentration of estrogen receptors (ERs) in the hypothalamus-preoptic area (HP) and anterior pituitary gland (AP) of chronically estradiol-treated ovariectomized rats. It has been reported previously that progesterone decreases the concentration of nuclear ERs in the uterus of chronically estrogen-treated rodents.

OVX rats were implanted sc with 5 mm Silastic capsules containing a 5% concentration of estradiol. One week later, they were injected sc with 5 mg progesterone or oil vehicle. Animals were killed 6 or 24 h later; HP, AP and uterus were dissected and assayed to determine both cytosol and nuclear ER concentrations. Confirming previous reports, by 6 h, uterine cytosol and nuclear ER concentrations were depressed by approximately 35%. Although progesterone was without effect on HP or AP nuclear ERs at this time, cytosol ER levels were decreased by 25% in HP and 14% in AP. At 24 h after progesterone injection, nuclear ER levels were decreased in all tissues while cytosol ER levels remained depressed. A study of the time course of progesterone's suppression of cytosol ERs indicated that this decrease is transient. Progesterone decreased the concentration of cytoplasmic ERs by 6 h, but by 48 h after progesterone injection receptor concentrations had recovered. Scatchard analysis confirmed that the decreased concentration of cytosol binding in HP was due to a decrease in the concentration of receptors rather than an alteration in the affinity of the receptors. Finally, as with nearly all of progesterone's neuroendocrine effects, the suppression of ER levels requires estrogen priming; in the absence of estradiol priming, ER levels were unaffected by progesterone injection.

These results suggest that under some conditions, progesterone decreases HP and AP estrogen receptor concentrations. Unlike progesterone's action in the uterus, the primary effect in the brain and pituitary gland seems to be on the cytosol receptor. Progesterone-inhibition of estrogen receptor levels may be involved in the neurochemical mechanism by which progesterone inhibits some of estradiol's influences on the brain and pituitary.

(Supported by BNS 13050 from National Science Foundation)

314.11 ANDROGEN AND ESTROGEN RECEPTORS IN FETAL RHESUS MONKEY BRAIN.
S. M. Pomerantz, T. O. Fox, R. W. Goy, S. A. Sholl*, H. Uno* and C. C. Vito. Wisconsin Regional Primate Res. Ctr., Univ. of Wisconsin, Madison, WI 53715. Dept. of Neuropathology, Harvard Med. Sch., and Dept. of Neuroscience, Mental Retardation Res. Ctr., Children's Hosp., Boston, MA 02115.

Sexual differentiation of rhesus monkeys has been found to be strongly influenced by actions of testosterone and dihydrotestosterone during prenatal development. In this study, we sought to demonstrate and characterize androgen- and estrogen-binding activities in fetal rhesus monkey brain, using the technique of DNA-cellulose chromatography. Both androgen and estrogen receptors adhere specifically to DNA-cellulose and each exhibits a characteristic elution pattern during chromatography.

Cytosol extracts of brain and anterior pituitary from male and female fetuses (ranging in age from day 135 to 162 postconception) were prepared using a buffer containing 50 mM Tris-HCl, pH 8.1 (21°C), 1 mM EDTA, 1 mM mercaptoethanol, 10% (v/v) glycerol, and 10 mM NaCl. Androgen- and estrogen-binding activities in cytosol extracts of hypothalamus/preoptic area/amygdala, anterior pituitary, and cerebral cortex were qualitatively similar to those found in other vertebrate species. Androgen receptor binds both dihydrotestosterone and testosterone and exhibits elution maxima at 120 - 140 mM NaCl. Estradiol binding activity exhibits elution maxima at 200 - 220 mM NaCl. Dihydrotestosterone binding activity was ~ 90% saturable at 10 nM and was reduced > 90% with 100-fold excess non-radioactive dihydrotestosterone. Estradiol-binding activity saturated at concentrations below 5 nM and was reduced > 90% with 100-fold excess non-radioactive estradiol. Concentrations of androgen and estrogen receptor were highest in the anterior pituitary, intermediate in the hypothalamus/preoptic area/amygdala, and lowest in the cerebral cortex. Comparisons of androgen- and estrogen-binding activities revealed that in the anterior pituitary, concentrations of estrogen receptor exceeded those of androgen receptor. Androgen and estrogen receptor concentrations were roughly equivalent in the hypothalamus/preoptic area/amygdala. In the cerebral cortex, androgen receptor concentration was greater than estrogen.

Collectively, these data demonstrate that in the fetal primate brain and anterior pituitary, distinct androgen and estrogen receptors are present which might mediate the action of gonadal steroids on sexual differentiation.

This work was supported by Grants RR00167, HD10818, MH21312 and HL27358 SCOR.

HORMONAL CONTROL OF BEHAVIOR II

315.1 DIFFERENCES IN RAT SUBSTRAIN SENSITIVITIES TO THE MATERNAL BEHAVIORAL EFFECTS OF OVARIAN STEROIDS AND OXYTOCIN.
C.A. Pedersen*, M. Johnson*, and A.J. Prange, Jr. (SPON: J.S. Hanker). Biol. Sci. Res. Ctr., Univ. North Carolina Sch. Med., Chapel Hill, NC 27514

We have reported that intracerebroventricular (ICV) administration of 400 ng of oxytocin (OXY) to virgin female Sprague Dawley rats from Zivic Miller Laboratories (ZMSD) 48 hr after ovariectomy (OVX) and sc injection of 100 µg/kg of estradiol benzoate (EB) produces a significantly higher incidence of full maternal behavior (FMB) within two hrs than ICV saline. Virgin female Sprague Dawley rats from Charles River Breeders (CRSDs) were tested for sensitivity to one of two doses of OXY (400 ng or 800 ng) 48 hr after OVX and sc priming with one of three doses of EB (100 µg/kg. 150 µg/kg or 200 µg/kg). Both doses of OXY were ineffective compared to saline in CRSDs primed with 100 µg/kg EB. In CRSDs primed with 150 µg/kg EB sc, 400 ng of OXY (3/12) was not, but 800 ng (8/13) was, significantly ($p < .02$, Fisher's exact probability) more effective than saline (1/11) in inducing FMB. In CRSDs primed with 200 µg/kg EB sc both 400 ng (7/14) and 800 ng of OXY (7/14) were significantly ($p < .04$) more effective than saline (1/11).

The spontaneous rate (SR) of FMB (the incidence within the first two hr of pup contact) and the mean latency (ML) of onset of FMB were compared in CRSDs and ZMSDs under widely differing ovarian steroid conditions. Animals were OVXed or sham-OVXed. Eight days later, each OVXed animal received sc one Silastic implant containing 2.2 mg 17 ß estradiol (E) or a blank. Ten days after OVX, each animal received sc three Silastic implants containing progesterone (P; 40 mg each) or blank implants which were removed 24 hr prior to initiation of pup contact on day 21 after OVX. Sham OVXed animals received blank implants as above. Results are summarized below:

	Sham OVX		OVX		OVX + E+P	
	SR	ML(days)	SR	ML(days)	SR	ML(days)
ZM	$8/20^{a,b}$	3.7(N=10)	$1/13^{b,c}$	6.1(N=7)	$12/17^{c,d}$	0.6(N=7)
CR	$0/17^{a,e}$	5.4(N=9)	2/18	4.1(N=10)	$5/16^{d,e}$	1.9(N=7)

$^{a,b,c,d,e}p < .05$, Fisher's exact probability, comparison of spontaneous rates (SR)

These data suggest that these two substrains of Sprague Dawley rats differ markedly in the influence of ovarian steroids on their rates of FMB response to rat pups and the ovarian steroid conditions that sensitize them to the maternal behavioral effects of OXY.

[Supported by NICHHD HD 16159 and NIMH MH-32316 and MH-22536 (AJP)]

315.2 LESIONS OF THE VENTROMEDIAL HYPOTHALAMUS INCREASE RATES OF ULTRASONIC VOCALIZATION IN MALE AND FEMALE HAMSTERS,
O.R. Floody. Dept. Psychology, Bucknell Univ., Lewisburg, PA 17837.

Past studies of animal sexual behavior have focused on stereotyped copulatory acts: mounting, intromission and ejaculatory patterns in males; lordosis responses in females. These studies have shown that levels of male and female copulatory behavior are decreased by lesions of the preoptic area-anterior hypothalamus (POA-AH) and the ventromedial hypothalamus (VMH), respectively.

We are interested in assessing the extent to which rules of brain organization based on studies of copulatory responses extend to other classes of sexual behavior. To this end, we have compared the effects of lesions on copulatory behavior and the rates at which hamsters produce ultrasonic (35kHz) calls that probably function to attract potential mates at an early stage in the normal sequence of mating behavior.

In this study, castrated, hormone-primed male and female hamsters were observed for rates of ultrasonic vocalization and levels of sex-typical copulatory behavior (intromission frequency in males, lordosis duration in females) before and after receiving sham operations or small, bilateral lesions of the POA-AH or VMH. The results confirmed previous work in showing that POA-AH lesions decrease intromission frequency whereas VMH lesions decrease lordosis duration. However, VMH lesions reliably increased rates of ultrasound production by males and females.

In males, these effects indicate that the neural circuits controlling copulatory and noncopulatory sexual behaviors are at least partly distinct. In contrast, the effects of VMH lesions on females identify an element of neural circuitry shared by mechanisms for lordosis and ultrasonic communication. Furthermore, the opposite effects of VMH lesions on calling and lordosis suggest a mechanism for the behavioral incompatibility of these responses: The suspension of ultrasonic calling that normally accompanies lordosis may be a consequence of an estrogen-dependent increase in VMH activity that simultaneously stimulates lordosis while inhibiting ultrasound production.

Supported by NIH grant MH33191.

315.4 SUPPRESSION OF CORTICOSTERONE SYNTHESIS ALTERS THE LOCOMOTOR BEHAVIOR OF HIPPOCAMPALLY LESIONED RATS. J. P. Ryan*, J. E. Springer*, J. H. Hannigan, and R. L. Isaacson (SPON. A. J. Schecter). Center for Neurobehavioral Sciences and Department of Psychology, SUNY-Binghamton, Binghamton, NY 13901.

In rats with hippocampal lesions, adrenalectomy significantly reduces the hyperactivity in the open field usually found after such lesions (Iuvone & Van Hartesveldt, Behavioral Biology, 19: 228, 1977). We investigated the pharmacological suppression of corticosterone synthesis with metyrapone, a drug that interferes with synthesis at the 11-β-hydroxylation step, in rats with extensive bilateral hippocampal destruction and control animals. In this way we could test each animal with usual glucocorticoid function and after glucocorticoid suppression following the drug treatment.

Rats were prepared with sham, cortical, or hippocampal lesions by aspiration. The animals were tested seven days after surgery. Following the determination of baseline behavioral levels, the animals were treated with either metyrapone or the metyrapone vehicle and observed for 10 minutes in a 16 hole open field apparatus. Further tests were given on the following days and included tests in which corticosterone (30 µg/100g bodyweight dose) was given before the metyrapone injections. Metyrapone drastically reduced the activity levels, rearing, and exploration of the hippocampally lesioned animals, and these decreases were restored to typically high levels by pretreatment with corticosterone. Sham control animals exhibited only a slight decrease in activity following corticosterone suppression by metyrapone, a decrease comparable to that reported in earlier studies for adrenalectomized rats relative to controls. Corticosterone pretreatment restored the activity levels of metyrapone treated intact animals to normal levels. The activity levels of cortical control animals were not affected by the treatments.

These results indicate that metyrapone pretreatment greatly reduces the hyperactivity induced by hippocampal damage and that the effect is due to the drug's impairment of corticosterone synthesis.

315.5 ACTION OF AN LHRH ANTAGONIST ANALOG ON SEXUAL PREFERENCE AND COPULATORY BEHAVIOR IN THE MALE RAT. C.A. Dudley, S.M. McCann and R.L. Moss. Physiology Dept., U.T.H.S.C.D., Dallas, TX 75235.

A potent antagonist analog, Ac-dehydro-Pro^1, pCl, D-Phe^2, D-Trp^{3,6} (LHRH$^-$), which has been shown to produce decrements in female sexual receptivity, was tested in the intact male rat for its influence on sexual preference and copulatory behavior. Third ventricular (V_3) cannulae were implanted in 13 sexually experienced Long-Evans male rats. One week after surgery, testing was begun and repeated at weekly intervals. Sexual preference was measured in a choice box apparatus, an "X"-shaped plexiglass structure lined with photocells along each of the four alleys. At the end of each alley was a box for housing choice animals. A wire mesh screen separated the choice boxes from the alleys. Frequency of entrance into each alley (F) as well as time spent in close proximity to the wire mesh at the end of each alley (T) was recorded. Two 15 min. baseline preference tests were conducted by placing a male into the center of the apparatus with no choice animals present. In this condition, F and T values for each alley were equal. Following each choice box test, the male rat was placed into a mating arena with a receptive female and time to first mount, intromission and ejaculation as well as number of mounts and intromissions to ejaculation were scored to ensure that cannulation did not disrupt copulatory behavior. After baseline testing, LHRH$^-$ (100 ng in 1 µl) or saline was infused into V_3 under ether anesthesia. Two hours later, choice box and copulatory behavior were measured as before. This time, however, a proestrus female (PF), an ovariectomized female (OF), or a stud male (SM) were placed in the outer boxes. The fourth outer box remained empty (EMT). Infusate treatment was reversed for the last test.

A matched pair t-test revealed that the time to ejaculation was significantly longer when LHRH$^-$ was infused ($\bar{x}$ = 20.9') than when saline was infused ($\bar{x}$ = 15.8') (t=5.11; p<.005). No difference in any other measure of copulatory behavior was significant. A repeated measure 3-way ANOVA (subject x choice x drug) was conducted on choice box T and F values. Significant subject, drug, subject x choice and subject x drug effects were obtained in the T analysis. Newman Keuls' test showed that more time was spent close to the PF than the SM or EMT and that more time was spent close to any choice animal than to EMT. Preferences in descending order were PF, OF, SM, and EMT. Infusion of LHRH$^-$ did not affect this preference order but it did reduce the overall amount of time spent close to choice animals. The frequency of entrance into the four alleys was similar for the LHRH$^-$ and saline treatments, indicating that LHRH$^-$ did not affect motor activity. Thus, prolongation of the time to ejaculation in LHRH$^-$ treated rats may be related to diminished interest in establishing contact. (Supported by NIH-HD11814).

315.6 ESTROGEN ACTIVATION OF COMPONENTS OF FEMALE SEXUAL BEHAVIOR IN SIX DAY OLD RATS. C. L. Williams. Dept. Psychol. Barnard College Columbia University, NY, NY 10027.

Current concepts of the process of behavioral sexual differentiation indicate that during the first 10 postnatal days estrogens act to sensitize or organize neurobehavioral systems necessary for the expression of adult sexual behavior. Presumably, the activational effects of this hormone on behavior cannot be demonstrated until later, after this early organizational action is complete. I report here data that questions the concept of separable periods of organizational and activational effects of steroids as it applies to female sexual behavior in the rat. Under certain conditions receptive (lordosis) and proceptive (ear wiggling) responses can be elicited in male and female rats as young as 6 days of age. Moreover, the display of both of these behaviors is facilitated by estrogen priming.

To study how hormones facilitate female sexual behavior, 4-day-old rats were injected with estradiol benzoate (0, 0.1, 1.0, 10, 100 ug/100g b.wt.) and returned to their dam. Four hrs prior to testing (40 hr after EB) pups were isolated and placed in an incubator (33° ± 2° C). At this time pups were given progesterone (0, 0.1, 0.5, 2.5 ug/100g b. wt.). Testing was accomplished by stroking pups' lower backs and flanks with a foam pad, and each of four trials was videotaped. Analysis of the behavior by an observer blind to the pups' treatment revealed the following:

In female infants EB increased the frequency, duration, and intensity of lordosis in a dose dependent fashion. For example, oil-treated females showed lordosis on 21.4 ± 10.1% ($\bar{X}$ ± S.E.M.) of trials; the response duration was 3.1 ± 1.2 sec and the mean intensity score was .6 ± .2. Comparable figures for females given 100 ug EB were 90 ± 10 % of trials, 6.4 ± 1.2 sec and 1.4 ± .2 intensity. Frequency of ear wiggling was also increased by EB treatment. When treated with a suboptimal EB dose (1 ug) progesterone facilitated responding in a dose dependent manner.

In male infants, EB did not increase lordosis frequency and intensity. This seeming lack of steroid facilitation in males seeemed to be due to the high baseline response of the oil-treated males. For example, control males showed lordosis on 50 ± 17 % of trials. This very high baseline frequency of response in control males may be due to their high levels of endogenous androgens. When males are castrated on the day after birth and then tested for lordosis on day 6, lordosis frequency is significantly lower than sham castrates and castrates given replacement testosterone.

These data are provocative in light of current knowledge of the maturation of steriod receptor function, and current theories about the process of sexual differentiation of brain and behavior.

315.7 DIFFERENTIAL EFFECTS OF KAINIC ACID AND IBOTENIC ACID LESIONS ON PSYCHONEUROENDOCRINE FUNCTIONS. T.R. King* and D.M. Nance. Dept. of Anatomy, Fac. Med., Dalhousie Univ., Halifax, Nova Scotia, B3H 4H7. (Spon: D.G. Gwyn)

The use of neurotoxic agents have provided new insight into the functions of the septal region (Nance, P.B. & B. 1983). We have replicated the psychoneuroendocrine effects of kainic acid (KA) lesions in the lateral septum (LS) and have extended these observations to include the medial septum (MS) and hippocampus (HP) and also have tested the effects of ibotenic acid (IBO) lesions. The effects of KA and IBO lesions in the LS and MS and HP on estrus cycles, ovarian compensatory hypertrophy (OCH), female and male sexual behavior, body weight (BWT), and estrogenic control of food intake were determined. KA (1µg/.5µl) was infused bilaterally into the LS (KALS) and HP (KAHP) and (2µg/1µl) unilaterally into the MS (KAMS). IBO (5µg/.5µl) was infused bilaterally into the LS (IBOLS) and MS (IBOMS). Electrolytic septal lesions (DCLS) and sham operated controls were included. KA and IBO lesions of the lateral septum resulted in complete bilateral loss of the LS; however, KALS lesions included CA3-CA4 cell loss in the HP. In contrast, KAMS and IBOMS lesions failed to produce obvious cell loss in the MS. KAHP lesions produced CA3-CA4 cell loss and a reduction in the size of the fimbria and rostral septum. Behavior and endocrine effects of these lesions were, relative to controls, KALS exhibited fewer percent days of vaginal estrus, increased OCH, increased BWT, attenuation in the anorexic effects of estrogen, and decreased female sexual behavior. KAMS exhibited a transitory increase in BWT and increased male sexual behavior. KAHP decreased female sexual behavior. IBOLS demonstrated a greater percent days of vaginal estrus and IBOMS exhibited a decrease in OCH. DCLS demonstrated increased female sexual behavior. Although KA and IBO lesions produced similar gross morphological damage, these neurotoxins had opposite effects on psychoneuroendocrine functions. Part of these alterations observed after KALS lesions may be attributed to loss of CA3-CA4 cells in the HP; however, the HP cannot be implicated in all psychoneuroendocrine alterations observed after KALS lesions. These results indicate that the septal region is involved in both facilitatory and inhibitory modulation of a variety of estrogen responsive processes and further suggest the differential involvement of the MS and LS in psychoneuroendocrine functions.

Supported by Medical Research Council

315.8 MEDIATION OF FEMININE SEXUAL BEHAVIOR BY A CHOLINERGIC MECHANISM: EVIDENCE FOR MIDBRAIN INVOLVEMENT. G. Richmond*, P. J. Barr*, and L. G. Clemens. Hormones and Behavior Laboratory, Michigan State University, East Lansing, MI. 48824

Feminine sexual receptivity (lordosis behavior) in rats, as in most mammals, is governed by the release of the ovarian hormones estrogen and progesterone. Recent work in this laboratory has demonstrated that this behavior may be mediated by a cholinergic mechanism in the central nervous system. The brain sites involved in this mechanism are as yet unidentified, and the goal of the experiment reported here was to investigate two brain areas implicated in the expression of lordosis to determine if they are involved in the cholinergic activation of the behavior.

Ovariectomized female rats of the Sherman strain underwent two simultaneous stereotaxic procedures: (i) bilateral electrolytic lesions of the midbrain central gray (CG), the ventromedial nucleus of the hypothalamus (VMN), or a sham lesion; and (ii) bilateral implantation of stainless steel cannulae terminating in the lateral ventricles. Animals were treated for three days with intramuscular injections of estradiol benzoate (0.175 ug). On the fourth day, animals were pretested for lordosis responses to mounting by a sexually vigorous male prior to intraventricular infusion of carbamylcholine chloride (carbachol; 0.25 ug in 0.5 ul/cannula), a cholinergic agonist. Two additional tests for lordosis were conducted at 5 and 20 minutes following infusion. Carbachol facilitated lordosis in females receiving sham lesions as well as lesions of the VMN. However, this facilitation was significantly attenuated in animals with CG lesions.

These results suggest that the CG plays an important role in the central cholinergic mechanism mediating lordosis. The precise nature of this role remains to be determined.

Supported by USPHS Grant HD-06760

315.9 ROLE OF THE OVARY AND ADRENAL IN THE ABBREVIATION OF ESTRUS INDUCED BY PACED COITAL STIMULATION IN THE RAT. M.S. Erskine* (SPON: M.J. Baum). Dept. of Nutrition and Food Science, M.I.T., Cambridge, MA 02139.

Cycling female rats, when allowed free access to and egress from a compartment containing a sexually active male, display clear patterns of activity (pacing) which govern the rate of cervical-vaginal stimulation received during mating. Previous work using partitioned test chambers in which the male is confined to one of two sides has demonstrated that paced coital stimulation results in a more rapid termination of the period of estrus than non-paced stimulation, even though the numbers of vaginal intromissions from males are similar in the 2 groups.

The present study examined whether the presence of ovaries and/or adrenals is necessary for the acceleration of estrus decline brought about by paced coital stimulation. At 2-week intervals, ovariectomized (ovx) and ovx-adrenalectomized (ovx-adx) females were given single injections of estradiol benzoate (EB; 8, 20, & 40 ug/kg) followed 48 hr later by 2 mg/kg progesterone (P) to induce sexual receptivity (lordosis). Four hr after the P injection, 3 groups of ovx and 3 groups of ovx-adx animals received paced intromissions, non-paced intromissions, or mounts-without-intromission. Tests for sexual receptivity were given 12, 15, & 18 hr after the initial treatment.

Pacing of coital stimulation by females during the treatment was not affected by EB dosage and was similar in the ovx and ovx-adx groups. In addition, longer periods of receptivity were displayed in all groups of ovx animals as the dosage of EB increased, while ovx-adx animals showed similar rates of estrus decline at all dosages. However, at none of the 3 EB dosages did paced ovx and paced ovx-adx groups show more rapid declines in estrous behavior than the non-paced or mounts-only groups. These latter results contrast with the strong effect of pacing on estrus duration in the intact cycling rat, and suggest that ovarian output in response to paced cervical-vaginal stimulation may contribute to the termination of estrus in the rat.

Supported by HD 16746 to M.S.E.

315.10 MIDBRAIN SINGLE NEURON ACTIVITY ASSOCIATED WITH ESTROGEN-PROGESTERONE INDUCTION OF LORDOSIS RESPONDING IN FEMALE GOLDEN HAMSTERS. James D. Rose. Department of Psychology, University of Wyoming, Laramie, WY 82071.

Previous microelectrode recording and lesion-behavior studies have demonstrated the importance of the midbrain, especially the tectum, in the sensorimotor control of golden hamster lordosis. The present work sought to identify, in freely behaving hamsters, midbrain neurons which might mediate sensory and motor control of the response and to observe ovarian hormone effects on these neurons as the lordosis-inducing hormone actions became apparent behaviorally. Ovariectomized hamsters were implanted with midbrain fine-wire microelectrodes for chronic single unit recording. A brief recovery period was allowed, during which a priming dose of estradiol benzoate (10 µg) was given. Approximately 40 hrs later, single unit activity was recorded immediately preceding and following subcutaneous injection of progesterone (500 µg). Recording was continued until after animals showed lordosis to manual stimuli, approximately 3 hrs later. Movement correlates of unit activity were monitored with an accelerometer mounted on the recording cable or with neck muscle EMG leads. Midbrain neurons with activity associated with locomotion or general movement were located in numerous regions, but especially the central and ventral tegmentum, whereas firing correlated with head movement was mainly shown by neurons in the deep laminae of the superior colliculus and adjacent tegmentum. Cells showing activity associated with motoric elements of lordosis were commonly found in the deep superior colliculus and underlying tegmentum also. In estrogen-primed hamsters, progesterone administration initiated a sequence of unit activity changes preceding and/or coinciding with the onset of lordosis responding. These changes included: 1) an elevation of dorsal midbrain neuronal activity, including the appearance of firing in previously inactive neurons; 2) enhanced responsiveness of some dorsal midbrain cells to lordosis-triggering types of somatic stimuli; and 3) in some ventral tegmental cells, a progressive decline of somatosensory responsiveness and locomotion-correlated firing. Recordings were also obtained following progesterone injections which were not preceded by estrogen priming or were given on the day after lordosis induction. These injections failed to produce lordosis and served as controls for progesterone effects not specific to lordosis induction. These control progesterone treatments altered the activity and responsiveness of some midbrain neurons but failed to produce the broad enhancement of dorsal midbrain activity and responsiveness which accompanied the lordosis-producing action of progesterone. Supported by N.I.H. Grant NS13748.

315.11 CONSTANT ILLUMINATION SUPPRESSES SEXUAL BEHAVIOUR IN MALE RATS Bryan D. Fantie[1], Richard E. Brown[1]*, and Will Moger[2]*. Departments of Psychology[1] and Physiology and Biophysics[2], Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1

Adult male Long-Evans rats (90 days of age) were housed under a 12:12 L:D cycle (LD, n=11); constant light (LL, n=13); or constant dark (red light) (DD, n=11) for 70-90 days. LL and DD rats were placed in running wheels in order to define their daily activity rhythms. Each male was given two 30 min sexual behaviour tests with a receptive female in dim red light. One test was in their active phase and the other in their inactive phase, separated by at least one week.

Over both tests, more rats housed in LD (75%) and DD (77%) mounted than did LL (55%) rats. Only 33% of the LL and 32% of the DD rats ejaculated. In contrast, all LD rats that mounted eventually ejaculated. Males in LD achieved significantly more ejaculations (sum of both tests, x=3.4) than males in the LL (x=1.5) or DD (x=1.2) conditions ($F=3.972$, $df=2,30$, $p<.05$).

One week after the second sexual behaviour test, each male had a blood sample taken by cardiac puncture under halothane anaesthesia. Blood samples were analyzed for testosterone (T) and prolactin (P) levels by radioimmunoassay. Males in DD had significantly higher T levels (x=7.13 ng/ml) than those in LD (x=3.10 ng/ml) or LL (x=2.88 ng/ml) ($F=25.486$, $df=2, 31$, $p<.05$). Males housed in LL had significantly higher P levels (x=56.0 ng/ml) than those in LD (x=33.09 ng/ml) or DD (x=39.28 ng/ml) ($F=3.854$, $df=2, 29$, $p<.05$).

The males were then sacrificed and the weights of their accessory sex organs taken. There were no differences among groups in weights of any organs: left and right testes; left and right epididymes, seminal vesicles; or ventral prostate.

Significantly more rats housed in LL had disrupted activity rhythms than those housed in DD ($p=.045$, Fisher exact test).

These results indicate that both LL and DD disrupt sexual behaviour in male rats and may do so through different neuroendocrine mechanisms.
(Supported by NSERC grant A7441 to REB and MRC grant MA5401 to WM.)

316.1 INPUTS TO MOTOR CORTEX: EPSPs FROM SENSORY CORTEX AND FROM THE FORELIMB. P. Zarzecki, D. Herman*, R. Kang* and M. MacGillis*. Dept. of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Cortico-cortical neurons with convergent sensory inputs project from sensory cortex to motor cortex (Zarzecki et al., Exp. Brain Res. 1982, 1983). It is not known how these cortico-cortical neurons might contribute to the formation of sensory properties of motor cortex neurons. Therefore, we compared cortico-cortical inputs with forelimb nerve inputs to motor cortex neurons. Microstimulation (3-40 uA) of area 3a of sensory cortex was used to evoke cortico-cortical EPSPs in motor cortex neurons of Nembutal anesthetized cats. Cortico-cortical EPSP latencies ranged from 1.3 to 9.1 msec. Eleven motor cortex neurons had cortico-cortical EPSPs with latencies less than 3.5 msec (2.3 msec $\pm$ 0.7 msec). These neurons were distributed between depths of 0.3 and 1.2 mm. This was expected from anatomical findings that cortico-cortical terminals in the motor cortex are not restricted to any particular cell layer.

To compare cortico-cortical EPSPs to inputs from the forelimb, two nerves were stimulated electrically: deep radial (a muscle nerve) and superficial radial (a cutaneous nerve). Individual motor cortex neurons received both cortico-cortical and forelimb inputs. In fact, all neurons with cortico-cortical EPSPs had inputs from all tested forelimb nerves. Forelimb nerve stimuli also evoked cortical surface potentials in area 3a which preceded the nerve-evoked EPSPs in motor cortex neurons. We compared 1) the delay between the time of arrival of the nerve-evoked surface potential in sensory cortex and the nerve-evoked EPSP in motor cortex with 2) the latency of the cortico-cortical EPSP for each neuron. This delay was usually longer than or was not significantly different from the latency of the cortico-cortical EPSP in the same neuron. This relationship suggests an "intracortical delay" in the relay of peripheral inputs to motor cortex. This interpretation is supported by the positive correlation found between these delays and the latencies of EPSPs evoked in the same motor cortex neurons by microstimulation of area 3a. Thus, the timing is appropriate for the cortico-cortical route to be contributing to the convergent somatosensory inputs received by motor cortex neurons. (Supported by the MRC of Canada.)

316.2 EXCITATORY AND INHIBITORY AFFERENTS TO RED NUCLEUS IN THE DORSAL-COLUMNS-LEMNISCAL SYSTEM. Y. PADEL* and T. JENESKOG* (SPON: ENA) Dept. of General Neurophys., CNRS, INP, F13277 Marseilles, France.

In the anesthetized cat, red nucleus cells are only slightly influenced in a non-discriminative way by exteroceptive stimuli. In opposition in conscious animals these cells are strongly reactive to somesthetic inputs : many natural stimuli -- gentle stroking of the fur, mechanic contact with the skin, joint rotations -- cause the firing of a burst of spikes in rubral cells. These projections often show restricted receptive fields and are somatotopically organized in the nucleus. Additionally the somesthetic map roughly superposates to the motor map of rubrospinal cells , with the forelimb represented dorso-medially and the hindlimb ventro-laterally in the nucleus. It was generally assumed that such responses were transmitted to the red nucleus through the cerebellum and/or the motor cortex. There are several arguments against this interpretation. Concerning the cerebellar loop, the VL nucleus of thalamus which receives the same cerebellar afferents as the red nucleus is only slightly or not at all influenced by natural stimuli. In concern to the cortical loop, the cortico-rubral synapses are on remote dendrites and not very effective.

The experiments were realized on acute , decerebrated and decerebellated cats , using intracellular recordings of rubrospinal cells. These cells were identified by their antidromic responses to stimulation of the rubrospinal tract in the spinal cord. Electrical stimulation of the forepaw was followed after a short latency by a mixture of long lasting EPSPs and IPSPs in the cells. Surface stimulation on dorsal columns with intensity as low as 20 μA induced , after shorter latency , similar compound post-synaptic potentials. Stimulation of lemniscus at bulbar level also gaves rise to PSPs of simple time course.

It is thus suggested that the rubro-spinal cells receive somesthetic excitatory and inhibitory inputs through the dorsal-columns of the spinal cord and the medial lemniscus after relay. It is proposed that this pathway might account for the somatotopically organized somesthetic responses demonstrated in the red nucleus in alert animals.

316.3 MULTIPLE PERCEPTUAL REPRESENTATIONS OF LIMB POSITION. J.R. Lackner and A. B. Taublieb*. Ashton Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, Ma. 02254.

Illusory motion of the unseen, restrained stationary forearm can be elicited by mechanical vibration of the biceps brachii or triceps brachii muscle. Such stimulation elicits a tonic vibration reflex, and the vibrated muscle contracts. The abnormally high level of muscle spindle activity that results is interpreted as stretch of the vibrated muscle and "referred" to the forearm, e.g. vibration of the biceps brachii causes apparent extension of the restrained forearm (Goodwin, G.M., McCloskey, D.I., and Matthews, P.B.C. Brain, 95, 705-748, 1972).

We have examined how visual information about limb position affects such vibration-induced illusions of limb movement. 18 subjects (Ss) participated in three conditions that were repeated twice, once in darkness and once under normal room illumination. The conditions included: a) S fixating his visible index finger, the rest of his hand and arm not being visible (FF), b) S fixating his index finger with his entire hand but not his forearm visible (FH), c) S attempting to fixate his unseen index finger (FUF). In FF and FH in the dark, S wore a surgical glove, the index finger or whole of which was coated with luminescent paint; in the FF and FH light conditions, S's finger or hand was visible in relation to external objects, the rest of his arm being shielded by a tube; in FUF in the light, the tube covered the entire forearm. In each condition, the biceps brachii of S's restrained right arm was stimulated with a physiotherapy vibrator (120 pulses/sec) for 90 sec. Ss estimated apparent seen and felt motion of their forearm according to a pre-practiced scale.

In FF and FH conditions in the dark, Ss saw their visible finger or hand move downward, an average of 22^{o} and 26^{o}, in keeping with the felt extension of their actually stationary forearm. In FF and FH conducted in the light, Ss felt their finger or hand move downward, 8^{o} and 3^{o}, but did not see them move; they felt the rest of their forearm move downward 17^{o} and 13^{o}, respectively. Eye movement recordings showed that the eyes steadily fixated the index finger in each condition. In the FUF conditions, Ss experienced extension of their forearm in both the dark and lighted room conditions, 22^{o} and 16^{o}. In the FUF dark (but not light) condition, llSs exhibited some pursuit eye movements as they attempted to fixate their unseen index finger.

We conclude: a) the seen and felt positions of the forearm can be dissociated. b) impossible limb configurations can be experienced with hand and forearm separately localized, c) visual motion of the physically stationary hand can be perceived although accurate visual fixation is being maintained. d) felt motion of the unseen stationary hand can elicit oculomotor pursuit.

Supported by NASA Contract NAS 9-15147.

316.4 KINESTHETIC "ENCODING" BY MOTOR CORTICAL NEURONS IN THE AWAKE CAT. W. Bedingham* and W.G. Tatton, Playfair Neuroscience Unit, University of Toronto, Toronto, Ontario, Canada, M5T 2S8.

Angular displacements of the cat forelimb, imposed by step-loads, evoke characteristic changes in the activity of different "subpopulations" of motor cortical neurons (MCNs). Correlations between the shape of average response histograms (ARHs) for the MCNs and the acceleration or velocity traces of the imposed movement suggested that four separate populations "encoded" velocity, acceleration, initial acceleration, and a combination of velocity and acceleration (North and Tatton, 1980).

The aim of this research was to determine whether individual parameters of imposed movements can be related to the responses of MCNs in area 4. Adequate examination of individual MCN responses required three different movement paradigms to be imposed on the limb. Accordingly step-loads, constant velocity ramps, and sinusoidal movements were used to construct the ARHs for each MCN. Furthermore, the information extracted from the imposed movement by single MCNs during individual trials was examined using stimulus-response matrices. "Clamping" movement velocity allowed measurements to be made on the reliability of single trial velocity "encoding" by individual MCNs. Finally, the anatomical loci of responding MCNs were determined using iron deposition and the Prussian blue reactions together with three-dimensional computer reconstruction of serial sections through the pericruciate cortex.

The ARHs for the majority of MCNs examined (N=150) were not found to be separable into four distinct response categories, but instead formed a continuum that could be described using third-order differential equations for the imposed angular displacements. That is, individual MCNs could be characterized by the weighted value assigned to the coefficients of position (θ) (recognizing the limitation that activity due to tension or position is often difficult to reliably separate), velocity ($d\theta/dt$), acceleration ($d2\theta/dt2$), and jerk ($d3\theta/dt3$, the derivative of acceleration). To date only two MCNs had prominent position coefficients. MCNs with predominant acceleration and jerk coefficients were usually found to have cutaneous receptive fields which contrasted with the finding that the receptive sites for MCNs with predominant velocity coefficients were most often in the deep tissues.

Initial analysis of MCNs with dominant velocity coefficients demonstrated that the velocity encoding reliability of single cells is relatively low compared to that reported for single mechanoreceptors. Specifically, the cells were found to encode a maximum of 2 bits of information. These findings indicate that populations of MCNs with similar movement parameter sensitivity would be necessary to characterize the imposed movement.

Computer models were developed to simulate the different movement paradigms (ie. step, ramp, sinusoid) and thereby quantify their position, velocity, acceleration and jerk coefficients. The responses of the MCNs were then constructed for the movements by accounting for inherent non-linear neural properties, conduction and synaptic delays, and convolutions of convergent synaptic inputs. The experimental and simulation results support the thesis that higher derivatives of movement are preferentially extracted by MCNs during imposed limb displacements and may be utilized in a predictive control system.

316.5 RESPONSE OF SOMATOSENSORY CORTICAL NEURONS TO VIBROTACTILE STIMULATION PRESENTED BEFORE AND DURING ACTIVE MOVEMENT. R. J. Nelson. Laboratory of Neurophysiology, NIMH, Bethesda, Maryland 20205.

The response of somatosensory cortical neurons to peripheral stimulation has been well documented for anesthestized and awake monkeys when the stimulated forelimb was immobilized to prevent movement. This study sought to determine: 1) the response to a vibrotactile stimulus when it served as a cue for the initiation of an active movement executed with the stimulated extremity, and 2) how the response to the cue might change in association with the task.

Rhesus monkeys flexed or extended their wrist in response to a vibrotractile cue (27-157hz low amplitude sine wave) delivered via the manipulandum after they maintained a fixed forelimb position for a randomized hold time (0.5-1.5s). The stimulus remained on until the animals' hand had moved 5 degrees around the wrist in this self-paced task. Of a total of 644 task related cells recorded in the pre- and postcentral cortex, 143 postcentral cells were chosen for this analysis because: 1) each had a short latency response (<30ms) to the stimulus onset, and 2) each showed further modulation related to some facet of the motor task. There was a marked decrease in the firing rate within 30ms after stimulus onset in 59 cells. Of these, 19% had cutaneous receptive fields (RFs), 13% with RFs in contact with the manipulandum and 6% with RFs located on other parts of the forelimb. Some 71% had well defined responses to joint manipulation. A few (10%) were sensitive to peripheral stimulation but no cutaneous field could be defined and were classified as having deep input. In no instance was there a decrease in firing rate in these same cells when a tactile stimulus was applied while the animal sat passively. A total of 84 cells responded to the vibrotactile cue with increased discharge within 30ms of stimulus onset, including 30 cells which were entrained to the stimulus frequency. This population includes 42% which had proprioceptive responses, 32% had cutaneous RFs, 14% had deep input and 12% were lost before testing. Of these 84 cells which exhibited increased discharge with the stimulus, 60% had a profound decrease in firing rate approximately 60-80ms prior to earliest detectable change in handle position. This decrease corresponds to the time at which neurons in precentral cortex exhibit peak activity during this task.

These observations suggest that cells in somatic sensory cortex are subjected to at least two types of influences. The first occurs just after stimulus onset and is present only preceding movement of the hand. The second occurs at approximately the same time as peak precentral cortex. Both types of influences may have important central, as opposed to peripheral, components as evidenced by their dependence on and correlation with active movement.

316.6 NUCLEUS GRACILIS PROJECTIONS TO THE MEDIAL AND DORSAL ACCESSORY OLIVES OF THE CAT: A RETROGRADE TRACING STUDY. H.H. Molinari. Department of Anatomy, Albany Medical College, Albany, N.Y. 12208.

It has been known for some time that the nucleus gracilis of the cat sends projections to both the medial and dorsal accessory portions (MAO and DAO) of the contralateral inferior olive. In order to differentiate which cells in n. gracilis project to MAO and to DAO, small injections of WGA-HRP were made in either 1) caudal MAO with no involvement of DAO or 2) ventrolateral DAO with no involvement of MAO. Care was taken to insure that the medial lemniscus was not damaged. The resultant tissue was treated with TMB and the locations of retrogradely labeled neurons in n. gracilis determined.

Following injections in MAO, labeled neurons were found along most of the length of the n. gracilis, from the obex to the caudal tip. They displayed no preferential location along the rostro-caudal axis but were always concentrated in the ventral portion of the nucleus.

Following injections in DAO, the number of labeled neurons in n. gracilis increased by an order of magnitude. In contrast to the MAO projection, there was a distinct preferential location of the DAO projection neurons along the rostro-caudal axis of n. gracilis. That is, approximately 70% of the labeled neurons were found in the transitional zone, which covers only 10% of the rostro-caudal extent of gracilis. Caudal to the transitional zone, a few labeled neurons were found scattered along the dorsal and ventral surfaces of the nucleus. There appeared to be a much stricter somatotopic organization in the DAO compared to the MAO projection. Injections in ventrolateral DAO only labeled neurons in n. gracilis while injections in MAO, regardless of how small, invariably labeled cells in both n. gracilis and n. cuneatus.

These data suggest that one subset of cells, those in ventral n. gracilis, projects to both MAO and DAO. This population gives rise to the entire projection to MAO but only a small fraction of the projection to DAO. Most of the DAO projection arises from the transitional zone.

(Supported by BRSG Grant SO-7RR05 394 and NIH Grant NS-17693).

316.7 CORTICAL AND TRIGEMINAL CONVERGENCE IN THE INTERTRIGEMINAL NUCLEUS. S. Landgren* and K.Å. Olsson* (SPON. H. Jasper). Department of Physiology, University of Umeå, S-901 87 Umeå, Sweden.

Previous observations have shown that the sensori-motor cortex exerts powerful effects on the monosynaptic jaw closing and disynaptic jaw opening reflexes (Olsson, K.Å. and Landgren, S., Exp. Brain Res., 39: 389, 1980). Contrary to earlier concepts of a rather stereotyped control we observed repeated phases of excitation and inhibition of both reflexes following cortical stimulation. The largest amplitude and shortest latency effects (2,5 ms) were evoked from the ipsilateral oral and perioral primary projection fields of areas 3a and 3b. In the present study the cortico-bulbar paths were further elucidated.

In the cat the cortical efferents do not make monosynaptic connections with the trigeminal motoneurons. Therefore, our interest was specifically directed to an analysis of the role of the interneurons surrounding the trigeminal motor nucleus (NVmt). Both ipsilateral cortical and trigeminal afferent inputs to the interneurons were sought in cats anaesthetized with chloralose. Unitary responses were recorded with glass micropipettes that were orientated using physiological landmarks. Mapping of cortical receiving areas was based on the recording of surface and unit potentials evoked by low threshold afferents. Cytoarchitectonic areas were determined and electrode tracks confirmed on serial histological sections.

Neurons receiving convergent inputs from descending cortical pathways and low-threshold primary afferents were identified in the lateral and ventrolateral borderzone of NVmt i.e. in the intertrigeminal area at the level of the middle and rostral thirds of NVmt. The most effective cortical points for evoking unitary discharge were located in the primary receiving fields of the whisker branch of the infraorbital nerve and the inferior alveolar nerve of areas 3a and 3b of the coronal gyrus. Input from all three dermatomes converged on the interneurones but one was always more effective than the others. The minimum latency of the response to cortical stimulation was 1-2 ms suggesting a monosynaptic path as the simplest route. Approximately 30% of the units were monosynaptically excited from the periphery (latency 1-2 ms) while 60% responded at a polysynaptic latency (>2 ms). The units fired bursts of up to 10 spikes at 400-900 Hz for the cortical stimulation and up to 15 spikes at similar frequency to the peripheral input.

Our current anatomical studies confirm that the interneurons in the intertrigeminal area complete the cortico-trigeminal pathway by projecting into the trigeminal motor nucleus. (Supported by Swedish Medical Research Council).

316.8 ROLE OF THE INFERIOR COLLICULUS IN THE INHIBITION OF ACOUSTIC STARTLE IN THE RAT. D. S. Leitner, M. E. Cohen* and M. C. Rosenberger*. Dept. of Psychiatry, Columbia Univ., New York City, NY 10032.

Sensory events which do not themselves elicit startle (prestimuli) have been found to modify the latency or the amplitude of the startle response, depending upon the amount of time by which the prestimulus precedes the startle-eliciting stimulus. In rats, prestimuli with lead times of 5 msec reduce startle latency but leave amplitude unchanged; lead times of 65 msec will reduce the amplitude of startle but leave the latency unchanged. These two effects are independent of each other and are cross-modal. In rats, both visual and auditory prestimuli can produce amplitude and latency reduction; latency reduction requires a somewhat more intense prestimulus than does amplitude reduction.

Recent work by Leitner, Powers, Stitt, and Hoffman (Physiol. Behav. 26: 259-268, 1981) has demonstrated that the lateral tegmental area of the midbrain is involved in startle amplitude reduction in rats. The present study investigated the role that the inferior colliculus, an auditory structure that projects on the lateral tegmental area, has in the amplitude reduction of startle in rats.

Fifteen rats were tested for startle amplitude reduction using both auditory (a 5 kHz, 70 dB tone) and visual (a light flash) prestimuli. The startle-eliciting stimulus was a 110 dB burst of white noise. Eight rats were then selected at random and given lesions of the inferior colliculus. The other 7 rats served as a normal control group. The surgical group was given two weeks to recover, and then all subjects were re-tested using a procedure identical to the one used previously.

Both groups showed startle amplitude reduction to both auditory and visual prestimuli on the pre-surgical test. After surgery, the group with inferior colliculus lesions showed a large increase in startle amplitude. They displayed no amplitude reduction to an acoustic prestimulus, but visual prestimuli were still effective in reducing startle amplitude in these subjects. The control group displayed normal amplitude and amplitude reduction to both types of prestimuli on the post-surgical test. Both groups showed latency reduction to the auditory prestimulus when the interstimulus interval was reduced to 5 msec. Thus, subjects with lesions were still capable of processing mild auditory stimuli. The effect of the lesions was specific to amplitude reduction by acoustic prestimuli.

These data demonstrate that the inferior colliculus plays a significant role in the inhibition of acoustic startle in the rat. The circuit for amplitude reduction by acoustic prestimuli ascends from lower auditory structures to the inferior colliculus before descending through the lateral tegmental area into the hindbrain.

316.9 ORGANISATION AND INTERRELATIONSHIPS OF FRONTAL, NIGRAL AND BULBOSPINAL AFFERENTS TO THE SUPERIOR COLLICULUS AND PERIAQUEDUCTAL GRAY MATTER IN THE CAT. R.-B. Illing* & A.M. Graybiel (SPON: A. Hofbauer), Dept. Psychol. & Brain Sci., MIT, Cambridge, MA 02139

We have studied the arrangement of inputs to the superior colliculus (SC) and the periaqueductal gray matter (PAG) arising in the frontal cortex (including the frontal eye fields),the substantia nigra and the bulbospinal junction (n. trigeminus and spinal cord),by combining anterograde axonal tracing and acetylcholinesterase (AChE) histochemistry in experiments on cats.

In transverse sections there are patches of high AChE activity separated by weakly stained zones in the upper part of the intermediate gray layer (level A). The patches (ca 200-500μm) can have complex forms, and sometimes fuse or form two tiers. Especially medially, variably dense AChE-positive radial extensions run down to a second discontinuous band of AChE activity deeper in the SC (level C), crossing a layer that has zones of weak AChE staining (level B). Radial AChE-positive streamers connect the C band with a distinct wedge of high AChE activity in the dorsolateral PAG.

There are striking correspondences between this complex histochemical pattern and the distribution of fiber endings labeled by frontocortical tracer injections. First, these fibers form patches which are aligned with AChE-patches in level A, and radial bands running between levels A and C in the two sets of sections are also in register. Second, there are prominent "holes" in level B labeling and these are aligned with gaps in AChE stain. Third, matching the AChE-pattern, frontotectal fibers continue ventrally in the SC and form a densely labeled wedge in the dorsolateral PAG.

The nigrotectal projection has a simpler form, being composed mainly of a single line of patches that are in register with the AChE-patches of level A. When these AChE-patches are two-tiered, the correspondence tends to be with the ventral tier. Nigrotectal fibers also terminate in the AChE-positive dorsolateral PAG.

In sharp contrast to these alignments, fibers labeled by injections of the bulbospinal junction tend to avoid the AChE-rich zones in the SC and PAG. The fibers terminate in well defined clusters in level A. These often fit into concavities visible in AChE-rich patches in adjoining sections, and when two tiers are formed by the AChE-patches, the labeled sites tend to fall in between them. There is an equally striking avoidance of the AChE-rich wedge in the PAG.

We conclude that the frontocortical and nigral projections to the SC, thought to be involved in oculomotor control, converge in discrete AChE-rich zones in the SC and PAG, and that these zones largely interdigitate with patches receiving ascending somatosensory (bulbospinal) input. It is as though these afferent systems avoid each others' target neurons, forming separate domains in the intermediate collicular layers and dorsolateral PAG.

Funded by Deutsche Forschungegemeinschaft & NSF BNS81-12125.

316.10 EFFECTS ON RUBRAL UNITS OF COOLING OR LESIONS OF N. INTERPOSITUS AND DENTATUS. D.E. Batson* and V.E. Amassian (SPON: R. Cracco). Dept. of Physiology, SUNY, Downstate Med. Ctr., Bklyn., N.Y. 11203.

Using the standard techniques of this laboratory, individual rubral neurons (RN) were recorded in awake cats, projection neurons (RPN) being identified by demonstrating antidromic invasion following stimulation of the contralateral medulla. Cooling n. interpositus with dentatus via an implanted probe for periods rarely exceeding one min in duration, abolished resting discharge in 71% and markedly diminished it in 16% of RPN (n=38). In 7 uninvaded RN, resting discharge was abolished in 43%. In addition, in 26 RPN and uninvaded RN tested during cooling, discharge evoked from physiological stimulation of the contralateral limb field was reduced or less often abolished in 92%.

In the awake intact cat, in the absence of overt movement, RPN predominantly have a resting discharge, e.g., at 15-60/sec. However, following a massive combined lesion of n. interpositus with dentatus, 76% of RPN (n=72) had no resting discharge. Most of the resting activity observed was accounted for by uninvaded RN. Driving by limb stimulation was usually absent, but was still prominent with face stimulation or moving the head. Nevertheless, contact placing (CP) in the forelimb returned within 1 to 2 days of the roof nuclear lesion, albeit delayed and grossly hypermetric. CP recovery was presumably due to alterations in extrarubral circuitry.

Although no change in the incidence of silent RPN occurred in the 3rd week (75%; n=28), an increased responsiveness to limb stimulation was observed. During the 4th to 6th weeks, the incidence of silent RPN fell to 44% (n=36) and responsiveness to limb stimulation further increased, but maximum evoked discharge rates were much lower than those observed in intact cats.

Thus in the awake, intact cat, cerebellar roof nuclear output is necessary for resting discharge in most RPN, notwithstanding the existence of other excitatory inputs (e.g., corticorubral; Tsukahara, N., 1978, J. Physiol., Paris, 74, 339-345). In addition, this cerebellar output is responsible for most of the RPN discharges following physiological limb stimulation; furthermore, "contact-locked" and "movement-locked" RN responses occurring during contralateral CP by the intact cat (Amassian, V.E., Batson, D., and Eberle, L., 1982, J. Physiol., 327, 62-63P) also depend on this cerebellar output. However, by four weeks after a lesion of n. interpositus with dentatus, remaining rubral inputs acquire an increased excitatory effectiveness, possibly via axonal sprouting (Tsukahara, 1978).

Aided by USPHS, NIH grants NS 10987 and 11219.

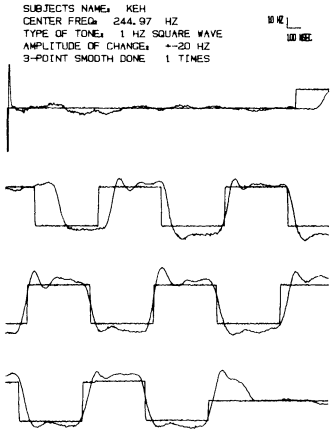
316.11 SINGLE UNIT ACTIVITY IN FRONTAL CORTEX OF MONKEYS LOCALIZING SOUND SOURCES. Eilon Vaadia, Moise H. Goldstein, Jr., Robert D. Hienz*, and Dennis A. Benson. Dept. of Biomedical Engineering, Johns Hopkins School of Medicine, Baltimore, Md. 21205

Previous work on the influence of behavioral tasks upon acoustically-evoked single unit activity (Benson, D. A., Hienz, R. D. and Goldstein, M. H., Jr., Neurosci. Abst. 5:43, 1979) has shown that while few neurons in the auditory cortex of monkeys show significant activity changes dependent upon whether or not the monkey responds to the sound source location, marked changes during such sound localization behavior can be seen in the dorsal periarcuate area, a region of frontal cortex which receives input from auditory cortex. In the present study microelectrode penetrations were made in the periarcuate region and in the dorsal and ventral prefrontal areas near the principal sulcus, with recordings obtained from 401 units in three monkeys. Five sound sources were equally spaced in the horizontal plane at midline (0°) and on either side of midline at 30° and 60°. The influence of sound localization behavior upon frontal units was examined by comparing such activity for three performance conditions: one which required the animals to respond to the location of a sound source (auditory localization), one which required the animals to simply detect sound onset regardless of location, and one which required animals to respond to the location of visual stimuli (visual localization). Most units in the periarcuate region responded weakly or not at all to acoustic stimuli presented when animals were not performing. When an animal actively localized stimuli, however, more than half of all units were highly "responsive", as indicated by strong, short-latency response components. Of these responsive units, the majority responded to both auditory and visual stimuli when animals performed the localization tasks. In the post-arcuate area, 90% of such units responded to both acoustic and visual stimuli during localization performance, while in the pre-arcuate area 47% responded to both types of stimuli during localization. Most of these auditory-visual responding units had similar spatial tuning characteristics for both acoustic and visual stimuli. In the post-arcuate area 85% of these auditory-visual units showed marked response differences, depending upon whether or not the animal was actively localizing stimuli. In the pre-arcuate area 44% of these units showed such behavior-dependent response differences. (Supported by NSF Grant No. BNS-76-81793)

316.12 VOCAL FREQUENCY TRACKING AS A PROBE OF THE MOTOR CONTROL OF THE LARYNX. H.B. Nudelman and D.B. Rosenfield*, Stuttering Center, Dept. Neurol., Baylor Col. Med., Houston, TX 77030.

Many studies have indicated (for review see Rosenfield Cur. Probl. Pediat. Vol. XII no. 8, 1982) the importance of the role of dynamic control of laryngeal muscle in the study of stuttering. A noninvasive measure of this control may be made by monitoring the fundamental frequency produced by a person while he is attempting to track a frequency modulated tone. The techniques used for analysis are analogous to those used in dynamic eye movement studies (Stark et al. IRE Trans. HFE-3, 1962).

Computer generated sine, triangle and square waves (.2Hz-4.Hz) as well as ramps ((.5-150)Hz/sec) are D/A converted and sent to a voltage controlled oscillator to produce frequency modulated tones in two free field speakers. The subject is asked to mimic these tones while his voice is detected with a throat mike and recorded along with the signal sent to the speakers. These signals are then A/D converted at 10 kHz and a cycle by cycle frequency is calculated using a slope and threshold crossing technique. This frequency data is then stored and plots like the one below are produced as an aid in modeling. As can be seen below there are both fast and slow pursuit movements in frequency. It is also apparent that after the first cycle the system shows the ability to predict the onset of upcoming cycles; notice the one at the end that is not present in the stimulus. Complete sets of data will be shown.



The support of the Perkins Foundation, the Bauer Foundation and the Ariel Medical Foundation is gratefully acknowledged.

316.13 STAPEDIUS MOTONEURONS IN THE CAT B.C. Fullerton, M.P. Joseph*, J.J. Guinan Jr. and B.E. Norris*. Eaton-Peabody Lab., Mass. Eye and Ear Infirmary, Boston, MA. 02114; EECS Dept., MIT

The location and number of neurons innervating the stapedius (ST) muscle of the middle ear has been studied by injecting the muscle with horseradish peroxidase (HRP). Control injections in the chorda tympani nerve, the facial nerve, the auricular branch of the vagus, the tensor tympani muscle and the cochlea were used to rule out contamination. The average number of ST cells for three cats was 1168. This is greater than the 199 seen by Lyon (1978), or the 668 seen by Shaw and Baker (1982), and is very close to the number of myelinated nerve fibers innervating the stapedius (1054) counted by Lyon (1979). In a cat in which HRP labeled ST cells were drawn, bleached (Adams, 1979), and restained with thionin, the ST cells in all regions contained medium-sized coarse Nissl granules. Several ST injected cats were processed for both acetylcholinesterase (AChE) and HRP, either in the same or alternate sections. Except in a few cases where the HRP labeling was too dense to see the cytoplasm, all HRP labeled cells were also AChE positive. Thus both Nissl pattern and AChE staining are consistent with all of the ST cells being motoneurons. Blevins (1964) estimated there are 1730 ST muscle fibers in the cat. If all of the HRP labeled ST cells are motor, then the ST innervation ratio would be about 1:1.6.

We have seen ST cells in perifacial and periolivary regions similar to those described by Lyon (1978) and Shaw and Baker (1982). It is interesting to ask whether these widely scattered cells constitute a uniform motoneuron pool. Based on location, we have divided ST cells into four groups: ventromedial (vm), ventral (v), and dorsal (d) perifacial (PF) groups, and a periolivary (PO) group. The largest ST group is the vPF with 43% of all ST cells, followed by dPF with 26%, PO with 23%, and vmPF with 9%. In transverse sections, the average major and minor axes of the cell bodies were 27 and 14 μm for the dPF group, 29 and 17 μm for the vPF group, 31 and 27 μm for the vmPF group, and 33 and 28 μm for the PO group. Each of the four groups was significantly different ($p<.01$) from the others in at least one size comparison. Caudal vPF cells are frequently fusiform, whereas vmPF cells are more multipolar. The vPF cells have many abutting dendrites suggesting the possibility of dendrodendritic synapses. Abutting dendrites are seen less frequently in dPF cells, and even less frequently in vmPF and PO cells. The above morphologic differences may reflect functional differences among ST groups.

Supported by NIH grants PO1 NS13126 and KO7 NS00621.

316.14 **FUNCTIONAL SEGREGATION WITHIN THE STAPEDIUS MOTONEURON POOL.** **M.P. McCue* and J.J. Guinan Jr. (SPON: N.Y.S. Kiang). EECS Dept., M.I.T.; Eaton-Peabody Lab., Mass. Eye & Ear Infirmary, 243 Charles St. Boston, MA 02114.**

The stapedius muscle in the middle ear contracts reflexively in response to intense sounds presented to either ear, and also contracts prior to the production of self-made sounds (e.g. vocalization, mastication, swallowing). Perhaps the most finely innervated muscle in the body, the stapedius receives about 1150 motoneurons through the facial nerve (Joseph et al., unpublished). Unlike those of most cranial muscles, the cell bodies of the stapedius motoneuron pool are not contained within a well-circumscribed nucleus, but are diffusely distributed in several large groups adjacent to the facial nucleus and within the superior olivary complex (Fullerton et al., Soc. Neurosci. Ab. 1983). In an attempt to discern the functional significance of this anatomical arrangement, the acoustically evoked surface electromyographic activity (EMG) of the stapedius was recorded from cats (anesthetized with Ketamine Hydrochloride). When the EMG activity of a single muscle was measured in response to ipsilateral, contralateral, and bilateral sounds (1 KHz tone bursts of 25 msec duration), a clear pattern of bilateral summation emerged: the effect of bilateral sound appeared to be the algebraic sum of the individual EMG responses to unilateral sound. After characterizing a muscle's EMG response to sound over a 50 dB range (usually up to levels at which the responses saturated), lesions were made at selected locations within the brainstem. To determine whether axons of stapedius motoneurons follow the course of the main facial nerve, the genu of the facial nerve was severed in the floor of the fourth ventricle. The contralaterally evoked EMG was virtually abolished in all cases (0-5% remaining), but surprisingly, much of the ipsilaterally evoked EMG response remained (25-75%). This differential effect on the EMG responses to sound in each ear was then studied by making unilateral, focal lesions (using R-F current) each of which destroyed part of the stapedius motoneuron pool. These lesions only changed the responses recorded from the muscle on the side lesioned, and produced three distinct results depending on their locations: (1) a significant drop in EMG elicited by contralateral sound with no change in EMG elicited by ipsilateral sound; (2) a significant drop in EMG elicited by ipsilateral sound with no change in EMG elicited by contralateral sound; and (3) no change in either EMG response. *No* focal lesion produced the simultaneous reduction of both ipsilaterally *and* contralaterally evoked EMG responses which would have been expected to follow the destruction of motoneurons receiving functional inputs from both ears *(binaural motoneurons)*. The fact that a focal lesion could eliminate a substantial subset of the motoneurons without affecting the muscle's saturated EMG response to sound, implies that at least part of the motoneuron pool was not acoustically active (it may, however, be activated by non-acoustic inputs). The results of focal lesions which reduced the EMG response when one ear was stimulated, but not when the other ear was stimulated, can then be explained by some combination of the following hypotheses: 1) The observed changes were due to the destruction of acoustic *inputs* to binaural motoneurons; the destroyed motoneurons were not acoustically active. 2) The lesions destroyed spatially segregated motoneurons which received functional acoustic input from only one ear *(monaural motoneurons)*. The monaural-motoneuron hypothesis is supported by the observed algebraic summation of EMG responses to bilateral sound, an effect consistent with the presence of two separate populations of monaural motoneurons, but less likely given a single population of binaural motoneurons. Both hypotheses appear to conflict with the conception (based on work with skeletal muscles) of a motoneuron pool in which each motoneuron receives functional projections from all inputs to the pool. Thus, the notion of a homogeneous motoneuron pool in which each motoneuron functions as part of a final *common* pathway does not seem to apply to the stapedius muscle of this anesthetized animal.

Supported by NIH grants P01 NS13126 and T32 NS07047

316.15 RESPONSES OF EPAXIAL MUSCLES AND MOTOR NERVES TO ELECTRICAL STIMULATION OF THE PUDENDAL NERVE IN THE RAT. M. S. Cohen, S. Schwartz-Giblin and D. W. Pfaff. The Rockefeller University, New York, NY 10021.

Under well-defined hormonal conditions, and when appropriately stimulated the female rat will exhibit a lordosis response. This stereotyped behavior is evoked by tactile stimulation of the flank/rump/perineal region and includes an exaggerated dorsiflexion of the spine. Sensory input for the reflex is primarily provided by the pudendal nerve which innervates the skin of the perineum (Kow and Pfaff, Brain Res. 101:47, 1976). Brink and Pfaff Brain Behav. & Evol., 17:1, 1980) have shown that the Lateral Longissimus (LL) and Transversospinalis (TS) muscles are important contributors to the dorsiflexion component of the lordosis reflex.

In order to characterize more fully the temporal properties and neural pathways involved in this reflex we have recorded the EMG activity in the LL and TS muscle while applying electrical stimulation to the pudendal nerves on both sides of the animal. We also recorded from the branches of the L_3, L_4 and L_5 muscle nerves which innervate LL.

Rats were anesthetized with urethane and the medial longissimus muscle was usually removed. Pudendal nerve stimulation was in the form of triple shock trains. The individual shocks were of 200 μs duration and were presented at a frequency of 500 or 800 Hz. The stimulus amplitude was increased gradually until contraction was visible in the abdominal region. In male rats the lowest threshold movements were visible as a scrotal retraction (cremaster reflex). Stimulus currents ranged from 10 to 750 μA and were usually about 200 μA. EMG was recorded with a silver ball electrode on the medial wall of the lateral longissimus muscle in the L_3-L_6 region and occasionally on the transversospinalis musculature. Muscle nerves were ligated and cut prior to recording.

In ovariectomized female rats, either estrogen pretreated or untreated and in male rats, the EMG response appears as a complex wave of activity beginning approximately 21 ms ($\bar{x} = 21.2 \pm 2.98$, N=12) after the end of the stimulus train and lasting for about 10 ms. This response is occasionally preceded by an early burst of activity at approximately 10 ms latency and followed, less frequently, by a discharge at 120 ms. The 21 and 120 ms responses are at times followed by broad waves of electrical activity which were probably due to contraction of the LL musculature.

PST histograms of the muscle nerve responses in female rats show an initial clustering of unit activity within 10 ms of onset of stimulation followed by a depression of activity which may last for 20 ms. Following this depressed period increased nerve activity resumes. Recordings from the muscle nerves typically show an overall increase in impulse activity during stimulation periods which returns to baseline after several hundred milliseconds.

316.16 CORRELATIONS WITHIN THE BALTIMORE EMG PAIN MODEL. N.R. Myslinski, J. Buxbaum, Dept. Physiology, Univ. of Maryland Dental School, and R. Parente, Dept. Psychology, Towson State Univ., Baltimore, MD.

The Baltimore EMG Pain Model is an experimental method of objectively determining levels of analgesia by analysing EMG signals from subjects suffering from chronic myofacial pain. The recordings are taken bilaterally from the masseter and anterior temporal muscles during 5 modes of activity: rest, swallowing, right side chewing, left side chewing, and clenching. This study was performed to determine which of these recording modes yields the strongest EMG/pain correlations.

Ten subjects reporting mild to severe myofacial pain participated. They were placed on a regime of a standard non-narcotic analgesic at the recommended dose for one week. Both subjective measurements using a visual analogue scale of pain severity, and objective measurements using the Pain Model were performed before and after treatment.

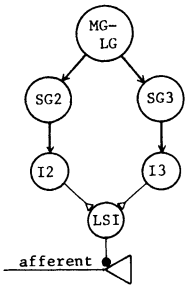
A computer based EMG-monitoring system analyzed both EMG frequency and amplitude. Several phi coefficients were computed on the data for each of the modes of activity. The phi coefficient is a measure of association that is specifically designed to quantify the relationship between two nominal variables (e.g., increases/decreases in pain rating from pre- versus post-test, and corresponding increases/decreases in EMG frequency and amplitude.

The results of the analysis indicate that the highest relationship between EMG and pain-ratings was in the rest position (0.66 for the amplitude and 0.66 for the frequency of the masseter EMG; 1.0 for the amplitude and 0.66 for the frequency of the anterior temporal EMG). The swallowing position was slightly less sensitive, and the remaining positions were relatively insensitive modalities. Further experimentation is necessary to determine if there are other positions which may be equally useful, relative to the rest position, for assessing the pain/EMG relationship or for predicting analgesic effectiveness. (Supported in part by grants from the Littman Co., the Sajik Corp, and the Upjohn Co.)

316.17 LOCAL AND DISTRIBUTED INTERNEURONS MEDIATING GIANT AXON-INDUCED PRIMARY AFFERENT DEPOLARIZATION IN THE CRAYFISH. M.D. Kirk and J.J. Wine. Dept. of Psychol., Stanford Univ., Stanford, CA 94305.

Any movement beyond the simplest reflex involves coordinated excitation and inhibition of large numbers of neural elements. Crayfish escape responses are useful models for studying the organization of inhibitory circuitry because a single impulse in a medial (MG) or lateral (LG) giant escape-command axon causes complex, widespread inhibition of central and peripheral elements. A particularly interesting form of command-evoked inhibition is presynaptic inhibition of the primary afferents, which protects the afferents from habituation during the tailflip (Krasne, F.B. & Bryan, J., Sci., 182:590-592, 1973). Primary afferent depolarization (PAD) of the mechanoreceptor axonal terminals is known to be correlated with presynaptic inhibition (Kennedy, D. et al., Sci., 186:451-454, 1974). PAD is delayed by about 10 msec after a giant axon impulse, so as to peak during the flexion movement of the abdomen.

We are investigating the interneuronal pathways that produce delayed, command-evoked PAD in the sixth abdominal ganglion. Simultaneous dual intracellular recordings (and dye fillings) of primary afferent terminals and interneurons in the pathway from giant axons to afferent terminals has revealed the following: (1) MGs produce PAD via interneurons they activate in anterior ganglia. (2) Four of these interganglionic interneurons that are fired by the giant axons (via segmental drivers; SG2, SG3) are identified (a pair of I2s and I3s) (Kramer et al., J. Neurophysiol., 45:550-573, 1981). Each can produce PAD of about 50% the amplitude of command-derived PAD, but they do so at long latency and often require two or more impulses, indicating summation of an interposed spiking interneuron. (3) The terminals of I2/I3 are bilateral but are not positioned so as to contact afferent terminals. (4) Local spiking interneurons (LSIs) have been found which fire at long latency to the giant axon impulses and which produce short latency (<1.2 msec), large amplitude PAD in afferent terminals. A minimal circuit to account for our results is shown.



Supported by NIH postdoctoral fellowship NS07074-01 (M.D.K.) and by NSF grant BNS 81-12431 (J.J.W.).

316.18 ANATOMICAL AND BEHAVIOURAL STUDY OF THE AFFERENTS TO THE PARAFASCICULAR NUCLEUS IN THE RAT. R. de Anda* and M. Garcia-Munoz. (SPON: R. Salceda). Dept. of Neurosciences, Research Centre in Cellular Physiology, U.N.A.M., Mexico.

It has been shown that stimulation of the parafascicular nucleus induces alterations in posture and balance maintenance, (Zainos et al. Exp. Brain Res) also, the afferents to this area in the rat have not been very well established. In an attempt to study the possible participation of parafascicular inputs, in the behaviour observed after its stimulation, we planned two experiments. First Evans Blue was injected into the nucleus (50-60 nl from a 10% solution). After a survival period of 4-5 days, the tissue was treated for fluorescence microscopy. Cells were observed in the substantia nigra reticulata, the red nucleus, the deep layers of the superior colliculus, the reticular formation of the pons, the central grey substance, the prepositus nucleus and the medial vestibular nucleus. Knowing the inputs, we proceeded in separate experiments, to stimulate the different areas with glutamic acid, (200 ng/ 0.3 ul) as a depolarizing agent. This stimulation followed a microinjection of 250 ng/ 0.5 ul of picrotoxin into the parafascicular nucleus. It was observed that the input which most influenced the parafascicular motor response, was the medial vestibular nucleus. It may be possible to conclude, that the vestibular input to the parafascicular nucleus, is modulating its output to other motor nuclei.

OCULOMOTOR BRAINSTEM MECHANISMS

317.1 EYE MOVEMENTS INDUCED BY PONTINE STIMULATION: INTERACTION WITH VISUALLY-TRIGGERED SACCADES. D.L. Sparks, L.E. Mays, J.D. Porter. Dept. of Physiology and Biophysics and Neurosciences Program, Univ. of Alabama in Birmingham, Birmingham, AL 35294

Monkeys compensate for unexpected perturbations in eye position produced by stimulation of the superior colliculus by looking to the spatial location of a visual target presented briefly prior to stimulation. Also, accurate compensation is observed in animals with bilateral transection of the ophthalmic branch of the trigeminal nerve - a procedure that, based upon anatomical findings, eliminates proprioceptive inputs from extraocular muscles. Collectively, these findings support models of the saccadic system which assume saccade targets are localized in spatial (nonretinocentric) coordinates and that a corollary discharge of the motor command provides a precise signal of eye position. This experiment assessed the ability of monkeys to compensate for changes in eye position produced by stimulation of sites other than the superior colliculus. Robinson's local feedback model of pontine circuitry predicts that animals will compensate for perturbations in eye position produced by stimulation of neurons providing an input to a "neural integrator" (NI), but fail to compensate for changes produced by excitation of neurons without direct or indirect input to the NI.

Animals compensated for stimulation of most, but not all, pontine sites at which long-lead or medium-lead burst neurons were encountered. When a visual target was flashed prior to pontine stimulation, the direction and amplitude of the stimulation-induced movement varied depending on a) target location and b) the interval between target onset and stimulation onset. Brief stimulation of the abducens nucleus produced abduction of the ipsilateral eye and a smaller adduction of the contralateral eye followed by an exponential return of the eyes toward their original orbital positions. In many cases abducens stimulation produced, perhaps through antidromically activated circuits, a small conjugate "step" change in eye position. Compensation was observed if the amplitude of the conjugate changes in position exceeded a threshold level.

Results are interpreted as follows: 1) Signals specifying the location of a visual target gradually build up during the reaction time interval and impose an obligatory delay prior to the next saccade trigger; 2) animals automatically compensate for perturbations in eye position that are produced by activity of the NI; and 3) the NI is anatomically close to the final common pathway.

Supported by EY01189 and P30 EY03039.

317.2 ACTIVITY OF MIDBRAIN NEURONS THAT ENCODE VERGENCE VELOCITY. Lawrence E. Mays and John D. Porter. Departments of Physiological Optics and Physiology and Biophysics and The Neurosciences Program, University of Alabama in Birmingham, Birmingham, Alabama 35294.

Extracellular single unit activity was recorded from midbrain areas in four rhesus monkeys. The animals were trained to look at visual targets displayed on far (72.5 cm distant) or near (25 cm distant) arrays or via a mirror stereoscope. Versional (conjugate) and vergence eye position could be manipulated by requiring the animals to fixate the targets precisely. The positions of both eyes were measured using the search coil technique.

A previous report (Soc. Neurosci. Abstr. 7, p. 133, 1981) described tonic convergence (divergence) neurons in the midbrain near the oculomotor nucleus. These units have a firing rate directly (inversely) proportional to the vergence angle regardless of the direction of conjugate gaze. We now report that some neurons in this area have a firing pattern which closely parallels the instantaneous velocity of ocular vergence. Most of these are convergence burst neurons which fire only for convergence. A few divergence burst neurons have also been recorded. The activity of all burst neurons leads vergence velocity slightly. Vergence burst neurons do not fire during conjugate movements.

Convergence and divergence burst neurons have the characteristics expected of inputs to a push-pull vergence integrator. Such an integrator is required by disparity-driven vergence because disparity signals a required change in vergence angle and not the vergence angle directly.

(Supported by NIH EY 03467 to L. Mays, EY 01189 to D. Sparks and Core Grant EY 03039.)

317.3 BRAINSTEM NEURONS RELATED TO ACCOMMODATION AND VERGENCE. Stuart J. Judge and Bruce G. Cumming* University Laboratory of Physiology, Oxford, England

We recorded from neurons in the vicinity of the oculomotor nucleus of the rhesus monkey while the monkey tracked a haploscopically presented target which appeared to move in depth (0.1 Hz, 4 or 8 diopters amplitude). The horizontal and vertical positions of both eyes were measured with magnetic search coils and, unlike previous investigators (Mays, Soc. Neurosci. Abstr. Vol. 7, p133, 1981 and Mays and Porter, Soc. Neurosci. Abstr., Vol. 8, p155, 1982) we monitored the accommodation of the right eye with an infra-red optometer. The haploscope was a complex device which permitted the target to be presented in a variety of different viewing conditions including (a) the normal combination of accommodation and vergence cues (except that no size change occurred), (b) 'prism viewing' in which the animal experienced the target as though a base-out prism had been placed in front of the left eye and (c) 'X2 gain viewing' in which the animal experienced the target as though her eyes were twice as far apart as normal (doubling the vergence to accommodation stimulus ratio). The purpose of the abnormal conditions (b) and (c) was to allow us to disturb the normal tight coupling between vergence and accommodation. We have recorded from 30 neurons near the oculomotor nucleus which are related to the near response in that their firing was approximately linearly related to vergence angle in the normal viewing condition, and equally strong whether the target moved towards the left or right eye (or towards the nose). Under the normal condition (a), the activity of most of these neurons was equally well related to accommodation. Initially, before we had trained the monkey on the (b) and (c) tasks, accommodation followed vergence under all conditions, but when a good accommodation target was present and behavioral pressure was applied, we were able to train the monkey to consistently perform the (b) and (c) tasks. We were then able to show that some at least of the neurons were not in fact related to vergence but to accommodation and that others were dependent in a complex way on both accommodation and vergence. It is therefore possible that the well-known synkinesis between accommodation and vergence is organised in the brain stem.

317.4 SACCADE-RELATED PAUSE-REBOUND CELLS IN CENTRAL THALAMUS OF MONKEYS. Madeleine Schlag-Rey and John Schlag. Dept. Anat. and BRI, Sch. Med., UCLA, Los Angeles, CA 90024.

Beside saccadic bursts, eye position and visually responsive neurons, the region of the thalamic IML (internal medullary lamina) contains neurons which show pauses and rebounds with all saccades. 58 IML cells of this type were studied in light and dark, in relation to large spontaneous saccades (e.g. 50°) as well as small fixation saccades (e.g. 2°). Eye position was measured with d.c. EOG (3 monkeys) or search coils (2 monkeys). To calibrate these signals, monkeys had been trained to fixate 1° visual targets.

1. Analysis of continuous sequences of saccades as a function of intersaccadic interval showed the significance of rebounds. Actually, the majority of cells (71%) discharged only after saccades. These "omnirebound" cells appeared to pause like true omnipausers (17%) only when the fast pace of saccades forced the clearing of rebounds with long time constant. Pause duration always encompassed the saccades. For cells which started pausing before saccades, the longest lead pause was around 80 ms. A greater variance between cells occurred with respect to rebound latencies (25 ms to 300 ms from saccade offset), although for each cell the latency was relatively constant (e.g. X = 25 ms ±5 ms). For a few cells, this latency was locked to saccade onset (fixed pause duration: 150-200 ms). Seven "late-pauser" neurons (12%) characteristically started pausing after saccades (pause duration: ± 200 ms).

2. For the majority of cells (72%) pauses and rebounds were identical in dark and light. In particular, rebounds were the same with steady gaze and drifting gaze in darkness. In a few cells, when all lights were suddenly turned off, rebounds were progressively attenuated until they completely vanished, several seconds later, too late for hypothesizing a direct visual sensitivity.

3. Little or no information about saccade parameters was encoded in rebound intensity. Instead, the pause-rebound cells appeared geared to send reliable "reset" and "ready" signals for the processing of the new visual information that is available after each movement of the gaze. The temporal spread of pauses and rebounds, small within cells, large between cells, may provide a mechanism to pace signals emitted from different regions. This hypothesis should be explored in view of the known connections of IML neurons with most visual areas (except 17), parietal and cingulate cortex, and frontal eye field.

(Supported by USPHS grant EY-02305).

317.5 PECULIAR NEURONAL ORGANIZATION OF THE CAT TROCHLEAR NUCLEUS. M. Shaw, R.F. Spencer and R. Baker. Depts. Pharmacol. & Physiol. Biophys., New York Univ. Med. Ctr., New York, NY 10016; Dept. Anatomy, Medical College Virginia, Richmond, VA 23298.

The extraocular nuclei in all mammalian species contain both motoneurons (Mns) and internuclear (Int) neurons. Recent use of sensitive HRP techniques has led us to conclude that the disposition of these neurons in the trochlear nucleus (TN) is more unusual than expected. Injection of HRP in the superior oblique (SO) muscle labeled an average of 1100 Mns in the $_{c}$TN; however, an average of 32 Mns was found in the $_{i}$TN. HRP in the tensor tympani (TT) muscle labeled up to 12 Mns in the $_{i}$TN. Mns of all sizes were observed but those to the $_{i}$SO distributed uniformly throughout the TN while those to the $_{i}$TT were localized mostly to a dorsal crescent-shaped cap of the TN. Axons of the Mns were observed to leave the nucleus with two different trajectories. Mns located caudal and lateral in the TN sent axons directly lateral to form the central IVth nerve tract near the base of the inferior colliculus. Axons of Mns located rostral and medial in the TN displayed initial trajectories directed towards the midline that curved ventrally, as far as the bottom of the MLF before hooking back up, into and around the TN, and then coursed laterally. Although Mns innervating the $_{i}$SO were largely of the latter type, their axons never crossed the midline but exited the brainstem laterally as a distinct fascicle, joining the passing IVth nerve to the $_{i}$SO muscle. Axons of Mns to the $_{i}$TT coursed caudally lateral to the MLF in superficial pontine areas before bending ventrally through the Vth motor nucleus to exit with the Vth nerve. An average of 30 Int neurons in the dorsal crescent part of the TN projected to the ipsilateral facial nucleus. Fewer (about 12, also in the crescent) projected to the area of the abducens and perihypoglossal nuclei. Uniqueness of the dorsal part of the TN was well illustrated by its anterograde labeling following HRP injection into the vestibular y-group. Since the y-group may be a contralateral excitatory saccular reflex pathway, Mns and Ints in this part of the TN may be involved with statolithic reflexes. By contrast, this role would not be likely for Mns innervating the $_{i}$SO. We conclude that, on the average, 10% of neurons in the cat TN do not innervate the $_{c}$SO muscle and that the dorsal rim is likely specific for a different role from the rest of the TN. Finally one wonders whether the Mns with dissimilar axon trajectories may be of different origin and even function. Supported by USPHS Grants EY02007, EY02191 and EY05404.

317.6 SEPARATION OF LARGE AND SMALL MOTONEURONS IN THE OCULOMOTOR NUCLEI OF THE MONKEY. J.A. Buettner-Ennever*, P. d'Ascanio*, H. Sakai* and H. Schnyder*. Inst. of Anatomy, University of Düsseldorf, Germany; Dept. of Physiology, University of Pisa, Italy; Dept. of Anatomy, University of Nagoya, Japan; Brain Res. Institute, Zürich, Switzerland. (SPON: B. Cohen).

Retrograde tracer substances were injected into individual extraocular eye muscles of monkeys. Labelled neurons were found in either the abducens or trochlear or oculomotor nuclei. The circumference of the labelled perikarya, seen to contain a nucleolus, was drawn on a computer terminal interfaced with a PDP11/20, which calculated the cell area and its location in the section. The distribution of neuronal diameters was clearly bimodal. The mean diameter of the large neurons was 28 µ and of the smaller neurons 17 µ. The differential location of the large and the small motoneurons populations was plotted for each eye muscle. There was a striking separation of the large and small cells in the oculomotor nucleus. The large motoneurons for IR, SR, MR and IO eye muscles lay within the confines of the classical oculomotor nucleus. Individual motoneurons subgroups for large motoneurons in the oculomotor nucleus correspond in general to the scheme of Warwick (1953), except for medial rectus which had a multifocal representation. The small motoneurons were distributed in a topographically organized fashion in a 300 µ band around the outside of the main nucleus. A less dramatic separation was also found in the trochlear and abducens nuclei. These results have important implications; first the oculomotor nucleus is larger than previously supposed: second the separation of large and small oculomotor neurons indicates a functional organization within the nucleus which may be related to the control of twitch and non-twitch fibers in eye muscles.

Supported by grants from the Deutsche Forschungsgemeinschaft SFB 200/A3 and EMDO, Zürich.

317.7 THE PROPERTIES OF ANTIDROMICALLY IDENTIFIED ABDUCENS MOTONEURONS IN THE ALERT CAT. J. M. Delgado-Garcia*, F. Del Pozo* and R. Baker. Dept. Animal Physiol., Faculty of Biology, Univ. of Seville, Spain; Dept. Biocybernetics, Faculty of Informatics, Polytechnic Univ., Madrid, Spain; Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

The activity of 53 antidromically identified abducens motoneurons with conduction velocities ranging from 13 to 70 m/sec were analyzed in alert cats during spontaneous and vestibular-induced eye movements. Numerical proportionality constants were obtained that could be correlated with the position (k) and velocity (r) profiles of eye movement. These measurements permitted direct assessment of a "size principle" rule contending that hierarchical organization of neurons within a pool is based on size alone with rank order functionally defined in terms of excitability. Slopes (k) of rate position values ranged from 2-17 spikes $\cdot$ sec^{-1}/deg and motoneuron recruitment from -19° to + 7°. A significant relationship was found between the eye position (threshold) for which a motoneuron began to fire and the slope of its rate position regression line (k). In addition there was a consistent relationship between the proportionality constant (k) and conduction velocity. Thus both recruitment and frequency potentiation in the cat abducens nucleus are, in part, dependent on morphological uniqueness intrinsic to cell size. However, the size principle was shown not to be an absolute criterion because the value of r varied significantly for individual neurons depending upon whether it was computed during saccades or the fast or slow phase of vestibular nystagmus. Individual variation ranged from 0.3 - 2.19 spikes $\cdot$ sec^{-1}/deg $\cdot$ sec^{-1} yet it completely averaged out over the population to 1.13 $\pm$ 0.45. As expected, r values were correlated, but less well, with conduction velocity leading to the conclusion that abducens motoneuron properties during recruitment and frequency potentiation depend on combined effects of (1) the distributed neuronal properties within the pool and (2) the pattern of synaptic effects from separate afferent systems. In addition to these observations we found significant hysteresis and effect of alertness in k and a position-dependent non-linearity of r. All motoneuron slopes (k) and 20% of the thresholds were significantly altered, producing large changes in time constants calculated from either individual r/k quotients or from phase lead analysis. Nonetheless, the above features did not alter the basic structure-function continuum positing a characteristic relationship between motoneuron sensitivity with position, velocity, threshold and axonal conduction velocity. Supported by USPHS Grants NS13742 and EY02007.

317.8 LOCALIZATION AND MORPHOLOGY OF GUINEA PIG EXTRAOCULAR MOTONEURONS AND INTERNUCLEAR NEURONS. C. Evinger and R. Baker. Dept. Neurobiol. & Behav., SUNY at Stony Brook, Stony Brook, NY 11794; Dept. Physiol. & Biophysics, NYU Med. Ctr., New York, NY 10016

This study examined the localization and morphology of guinea pig extraocular motoneurons and oculomotor and abducens internuclear neurons using extra- and intracellular horseradish peroxidase (HRP) staining. Retrograde transport following injections of HRP into single extraocular muscles localized the eight pools of motoneurons. The neurons in the abducens and accessory abducens nuclei were found to innervate the ipsilateral lateral rectus and retractor bulbi muscles, respectively. Injections into the superior oblique labelled motoneurons in the contralateral trochlear nucleus and a few neurons in the ipsilateral trochlear nucleus. The medial rectus, inferior rectus, and inferior oblique muscles received innervation from neurons in the ipsilateral oculomotor nucleus. The superior rectus and levator palpebrae muscles derived their input from the contralateral oculomotor nucleus. While idiosyncratic for the guinea pig, one aspect of the organization of oculomotor motoneuron pools differed from frontal eyed animals. As in the rabbit and rat, the guinea pig superior rectus and levator palpebrae motoneurons resided in the lateral portion of the nucleus. In cats and monkeys, these motoneurons straddled the midline.

On the average the intracellularly stained motoneurons had six primary dendrites with an overall dendritic spread of 600-800 microns. Each neuron sent dendrites over 1/3 to 1/2 of the nucleus and into the medial longitudinal fasiculus and central gray. Only a few motoneurons possessed axon collaterals and these collaterals usually terminated in the midline near the exit of the third nerve from the brain. In comparison, cat oculomotor motoneurons had six to eight primary dendrites with an overall dendritic spread of 1 mm. Most cat motoneurons exhibited axon collaterals but the collaterals always terminated within the oculomotor nucleus.

Extracellular deposits of HRP into the oculomotor and abducens nuclei demonstrated the location of the internuclear neurons. Abducens internuclear neurons capped the ventral edge of the abducens nucleus and projected to the medial rectus subdivision of the contralateral oculomotor nucleus. Oculomotor internuclear neurons projecting to abducens were located primariarly around the ventral aspects of the oculomotor nucleus. In comparison, cat abducens internuclear neurons were contained within the abducens nucleus and oculomotor internuclear neurons in the cat and rabbit resided within the oculomotor nucleus and overlying central gray. Supported by Grant EY04829 NEI.

317.9 MORPHOLOGY AND SYNAPTIC CONNECTIONS OF PHYSIOLOGICALLY-IDENTIFIED SECOND-ORDER VESTIBULAR AXONAL ARBORIZATIONS RELATED TO CAT OCULOMOTOR AND TROCHLEAR MOTONEURONES. R.F. Spencer and R. Baker. Dept. Anat., Med. Col. Virginia, Richmond, VA 23298, and Dept. Physiol. Biophys., New York Univ. Med. Ctr., New York, NY 10016.

Reciprocal synaptic connections of contralateral excitatory (Vc) and ipsilateral inhibitory (Vi) second-order vestibular neurones with motoneurones (MNs) in the oculomotor and trochlear nuclei provide the physiological basis for the vertical vestibulo-ocular reflex. The morphology and synaptic connections of second-order vestibular axonal arborizations, identified physiologically and stained by intraaxonal injection of horseradish peroxidase, have been examined in relation to inferior rectus (IR) and superior oblique (SO) MNs by light and electron microscopy.

Both Vi and Vc axons exhibited variations in the pattern and extent of arborization in relation to IR and SO MNs, with either approximately equivalent or weighting of synaptic input to only a small proportion of one or the other population of MNs.

Synaptic contact zones of Vi boutons were characterized by an accumulation of pleiomorphic synaptic vesicles along the presynaptic membrane and an inconspicuous postsynaptic membrane densification. In most cases, a single Vi bouton contacted only one MN soma or proximal dendrite, but at least 3 and as many as 9 separate synaptic contact zones were established with the same postsynaptic profile. In a few instances, however, one or more branches of a single Vi axon, each forming two or more boutons, established multiple synaptic contacts with the soma and proximal dendrite of the same MN.

Most Vc boutons established synaptic connections with medium- or small-calibre dendritic shafts or occasionally with dendritic spines. Synaptic contact zones were characterized by an accumulation of spheroidal synaptic vesicles along the presynaptic membrane and a prominent postsynaptic membrane densification with occasional subjunctional dense bodies. Most Vc boutons established synaptic contact with single dendritic profiles, although, in contrast to Vi boutons, only one synaptic contact zone was associated with each postsynaptic profile. Occasionally, however, one or more branches of a single Vc axon gave rise to a series of several boutons that all contacted the soma of the same MN.

These findings indicate that two important features that presumably relate to the efficacy of vestibular inputs to extraocular MNs are (1.) the mode and pattern of synaptic connectivity of a single vestibular axon with the target MNs, and (2.) the number of target MNs with which a single vestibular axon is in synaptic relationship. The synaptic arrangements of Vi and Vc axons may provide the basis for the presynaptic control of MN recruitment in the vestibulo-ocular reflex.

Supported by USPHS Research Grants EY02191, EY02007, NS13742.

317.10 PERIHYPOGLOSSAL AND VESTIBULAR PROJECTIONS TO THE SUPERIOR COLLICULUS IN THE CAT. M.T. Stechison*, J.A. Saint-Cyr and S.J. Spence*. (SPON: R.D.G. BLAIR) Playfair Neuroscience Unit, Toronto Western Hospital, and Departments of Surgery, and Anatomy, University of Toronto, and Toronto Western Hospital, Toronto, Ontario.

In view of the recognized role of the superior colliculus (SC) with regard to eye and head movement, and following recent physiological studies, inputs from the vestibular complex, and adjacent cell groups to the SC were sought with axonal transport techniques.

Injections of WGA-HRP (0.05-0.1 µl, 0.5-2.0 g%) were made in the vestibular nuclei of 25 adult cats under pentobarbital anesthesia. Two cases with injections including nucleus intercalatus (INT), and prepositus hypoglossi (PH) displayed anterograde labelling in the intermediate and deep layers of the contralateral superior colliculus (SC).

In 2 additional cats, WGA-HRP injections centred rostromedially, and rostrolaterally and extending throughout the rostral 2/3 of the SC were made (0.1 µl, 2.0 g% each). Retrograde labelling appeared in cells of contralateral INT in the former, with fewer cells in the latter being equally divided between PH and INT. To elucidate the topography of the projection, smaller volumes (0.02 µl) of WGA-HRP were placed in specific quadrants of SC. Labelling was seen in INT, PH, and medial vestibular nucleus, with two caudomedial injections favouring the contralateral PH, while a caudolateral case favoured INT. Further topographical information will be obtained from these cases, and from autoradiographic material in preparation.

Recent anatomical studies in the monkey (D.W.F. Schwartz, personal communication) suggested the presence of a direct projection from the C2 ganglion (DRG) to INT, the descending vestibular nucleus (DVN), and vestibular subgroup x. Large injections were placed in the C2 DRG of 3 cats. Of interest in the present context, there was labelling of ipsilateral INT and posterior DVN in one, bilateral INT in a second, and no label in a third.

The input to x is thought to be exclusively from neck proprioceptors and there is ample evidence of vestibular nuclear input to INT and PH. Thus, these latter two structures may be centres for the integration of neck proprioception, and vestibular information to be relayed to SC.

Supported by Medical Research Council of Canada grant MT-72[illegible] to J.S.C.

317.11 PROJECTIONS OF THE VENTRAL MIDBRAIN TEGMENTUM TO THE PERIAQUEDUCTAL GRAY (PAG) IN RABBIT: VISUAL-OCULOMOTOR PATHWAYS FROM THE MEDIAL TERMINAL NUCLEUS (MTN) OF THE ACCESSORY OPTIC SYSTEM. [1]Y. Torigoe*, [2]R.H.I. Blanks, [1]R.A. Giolli, [1]J.H. Fallon (SPON: [1]D.D. Williams). [1,2]Dept. Anatomy and [2]Surgery (Otolaryngology), Univ. Calif. Irvine, Irvine, CA 92717.

Previous studies in a variety of non-mammalian species have shown a direct connection with the oculomotor nucleus (3n). However, this projection has not been demonstrated in mammals. In the present study, discrete injections of ^{3}H-leucine were made into the MTN and adjacent structures and the tissue processed using the standard light autoradiography (ARG). The ARG materials revealed a strong bilateral (mainly ipsilateral) projection to the medial nucleus of the PAG (also called the supra-oculomotor PAG) adjacent to the lateral and dorsolateral portions of the 3n. However, a thin unlabeled zone clearly separated the terminal fields from the borders of the 3n. This area of PAG has been implicated in oculomotor function because: 1) it receives input from the superior colliculus and frontal eye fields; 2) it contains oculomotor internuclear neurons projecting to the abducens nucleus and 3) previous reports in mouse and cat and our preliminary data in rat and rabbit show dendrites of the 3n motoneurons extending into the area of the PAG covered by the terminal fields.

To confirm and clarify the location of neurons projecting to the PAG, retrograde transport of HRP was used. Labeled neurons were found in the MTN, the ventral tegmental area (VTA) and a portion of the VTA that we term the visual tegmental relay zone (VTRZ). The latter is particularly important considering the strong projection to the VTRZ from the contralateral MTN (Giolli, unpublished observations) and that these neurons project to the dorsal cap of the inferior olive (IO). These data from anterograde and retrograde studies suggest that the MTN can influence the activity of some oculomotor neurons (those with dendrites in PAG) by way of ipsilateral projections to the PAG and through a crossed MTN-VTRZ-PAG pathway.

Lastly, to test the possibility of collateralization of these neurons to the PAG and IO, double-labeling, using true blue/granular blue and nuclear yellow, was used in combination with glyoxylic acid-induced catecholamine fluorescence. The results confirm the HRP data, showed no collateralization of the VTRZ neurons and demonstrated that neurons projecting to PAG and IO are non-dopaminergic. Therefore, we conclude that the most direct and strongest visual pathway arising from the MTN to the dendritic extension of 3n motoneurons is through the VTRZ and the PAG.

317.12 PGO WAVE AMPLITUDE AND EYE MOVEMENT DIRECTION:EVIDENCE FOR COROLLARY DISCHARGE DURING REM SLEEP IN THE CAT. A.P. Monaco*, H.A. Baghdoyan*, J.P. Nelson, J.A. Hobson. Laboratory of Neurophysiology, Department of Psychiatry, Harvard Medical School, 74 Fenwood Road, Boston, MA 02115.

Ponto-Geniculo-Occipital (PGO) waves were recorded bilaterally from the LGB and visual cortex (CTX) in cats during REM sleep and waking (W). PGO wave amplitudes were significantly related to the horizontal direction of eye movements in REM sleep but not in W. In REM sleep, leftward eye movements are associated with larger amplitude (primary) waves in the left LGB and CTX and smaller amplitude (secondary) waves in the right LGB and CTX. Similarly, rightward eye movements are associated with right primary and left secondary waves.

Four male cats were implanted stereotaxically with teflon-coated stainless steel macro-electrodes bilaterally in the LGB and visual CTX (Area 18) to record PGO waves. EEG, EOG and EMG electrodes also were implanted. Recordings were done on an ink-writing polygraph during REM sleep and W states.

Polygraphic recordings during REM sleep consistently showed PGO primary waves in the CTX and LGB on the side ipsilateral to the direction of the corresponding eye movement. During REM sleep, leftward eye movements (N=370) produced primary waves almost exclusively in the left CTX (93.8%) and rightward eye movements (N=370) in the right CTX (92.2%). In W, however, CTX and LGB primary waves occurred almost equally on the left and right side regardless of the direction of the eye movements.

Mean amplitudes (in mm) for right and left CTX and LGB waves were calculated for each eye movement direction. In REM sleep, leftward eye movements produced significantly greater PGO wave amplitudes ($p<.001$, paired t-test) in the left CTX (20.3) vs right CTX (15.8) and left LGB (15.7) vs right LGB (9.2). Similarly, rightward eye movements produced significantly greater wave amplitudes ($p<.001$) in the right CTX (20.6) vs left CTX (15.9) and right LGB (14.3) vs left LGB (10.6). In W, there were no significant differences in left vs right wave amplitudes for either direction of eye movements.

These results demonstrate that in REM sleep, but not in W, the sensory visual system receives information concerning the direction of upcoming eye movements via the laterality of PGO primary waves. This evidence strongly suggests that PGO waves in REM sleep may represent corollary discharge signals that convey information about the direction of eye movements generated by the brainstem oculomotor system to the visual system at the thalamic and cortical levels.

Supported by grant MH 13923.

317.13 GRASPING IN THE PIGEON: BEHAVIORAL ANALYSIS OF A VISUOMOTOR CONTROL SYSTEM. J. R. Deich*, B. G.Klein and H. Philip Zeigler. Dept. of Ornithology, American Museum of Natural History and Biopsychology Program, Hunter College (CUNY), New York,N.Y. 10021

Considered as an effector organ, the avian beak has functions analogous to those of the hand. Grasping is integrated into the pigeon's pecking response system and involves the coordinated activity of the upper and lower beaks. Previous studies have shown that beak opening begins 20-30 msec. after the start of pecking. The amount of beak opening (gape) immediately prior to contact with the seed, varies directly with seed size and probably involves feedforward control. A quantitative analysis of grasping in the pigeon suggests it may be a useful model system for the study of vertebrate motor control mechanisms.

Gape was transduced using a Hall-effect device mounted on the upper beak which measured the strength of the magnetic field generated by a samarium-cobalt magnet on the lower beak. Variations in interbeak distance (gape) generated a voltage proportional to distance which was monitored by a microprocessor during pecking at a series of five standard size food pellets (range 3.2 to 11.1 mm.). Gape functions derived in this manner were approximately linear and adjustment of gape to pellet size was quite precise. Analysis of the behavioral mechanisms involved in generating gape functions reveals that both the velocity and the duration of beak opening vary with pellet size. The duration component reflects primarily the contribution of head movement and is not effected by section of beak motor nerves. We conclude that grasping in the pigeon involves the operation of two distinct effector systems one controlling head movements (neck motoneurons) and one controlling beak movements (jaw motoneurons). The coordinated operation of these systems in the pigeon's grasping resembles, in many respects, that of the transport and manipulation components of visually guided grasping in primates. Supported by Grants MH-08366 and Research Scientist Award MH-00320.

317.14 FROG PREY ORIENTING: VARIATIONS WITH STIMULUS DISTANCE. S.K. Kostyk, A. Reyes*, L. Zwanziger*, and P. Grobstein. Dept. Pharm. Physiol. Sci., Univ. of Chicago, Chicago, Ill. 60637

Prey orienting movements in the frog, Rana pipiens, vary with stimulus distance. D. Ingle reported that as distance increases there is a fairly abrupt switch between two qualitatively different motor patterns. For near stimuli the triggered output is a direct snap, including a tongue flip. For more distant stimuli it is a forward hop, without a tongue flip. We observed responses of frogs to live mealworms presented in front of the animals at various distances and confirm the existence of a switch between motor patterns which occurs at a particular distance. We also found that snap amplitude is graded with stimulus distance for nearer distances and that hop amplitude is graded with stimulus distance for greater distances. The latter indicates that the motor switch occurs at a distance which is within, rather than at the limit of, the range over which frogs can discriminate distances.

We determined the distance at which the motor switch occurs in frogs varying from 3 to 9 cm. in snout to vent length. There was a clear correlation between distance and body size with the former being on the average twice the latter. This indicates that some variable related to body size is important in determining the distance at which the switch between motor patterns occurs.

We studied distance-dependent behavior in animals following section of one optic nerve. Each of four animals showed normal variations in snap amplitude with stimulus distance. All four also exhibited switching between snapping and hopping at a well-defined distance, as previously reported by Ingle. We conclude that binocular cues are not essential for these aspects of the distance-dependent variation in prey orienting. Similar observations were made on frogs enucleated prior to metamorphosis. This indicates that binocular experience is also not essential.

In three of the four nerve-sectioned frogs, the distance at which the motor switch occured decreased after optic nerve section. In the fourth it increased. The latter observation indicates that the switch does not necessarily occur at a distance corresponding to the maximum possible snap amplitude. The fact that in all four animals the switch distance changed while snap amplitudes remained normal indicates that the switch distance can be altered without generalized changes in distance judgement.

Adequate models of the neuronal organization underlying orienting movements in the frog must account not only for movement direction but also for variations with stimulus distance of the kind described here. It seems clear that variables in addition to the particular retinal region activated must be involved in determining the output associated with a stimulus at a given location in space.

Supported by PHS EY-01658 and NSF BNS-7914122

317.15 ANATOMY AND FUNCTION OF FROG'S TECTAL EFFERENTS. D. Ingle. Dept. of Psychology, Brandeis University, Waltham, MA 02254.

Anterograde labelling with HRP demonstrates two major routes from tectum to the brainstem: (1) a crossed "tectospinal" path which terminates mostly in the caudal medulla; (2) an uncrossed "tectobulbar" path reaching the caudal medulla. Retrograde label from HRP implants in these respective regions reveals cells of origin of these tectofugal axons. All cells of the tectospinal system are in the contralateral tectum and all have visible dendritic ramifications only at the uppermost tectal lamina, where retinal class-1 and class-2 axons terminate. By contrast tectobulbar cells are located in the ipsilateral tectum, and all have visible dendrites ramifying below the class-2 zone. Some of these ipsilateral cells have dendrites branching within both class-2 and class-3 laminae. Of those cells whose processes are clearly labelled, there appears to be no overlap in morophologies of contralateral and ipsilateral systems.

Selective cuts of the crossed projection at the midline (or unilaterally in the medulla) abolish turning toward prey moving within the lateral field, although the lunge and snapping sequence remains intact for nearby prey. Jumping away from looming threat stimuli also appears to be mediated by the ipsilateral tectal projections. Therefore the tectospinal path is required for localization but not for identification of prey stimuli. By contrast, a hemisection of the brainstem just caudal to one tectum produces the opposite syndrome: such frogs fail to turn away from approaching threat, and fail to snap at nearby stimuli via the contralateral eye although they are able to orient accurately toward more distant prey objects. Partial transections of the tectobulbar pathway at the anterior medulla level can result in selective loss of either snapping or threat-avoidance turns. The present study, together with earlier behavioral and physiological data, indicate that tectofugal cells with specific behavioral functions have different modes of access to classes of retinal axons.

CEREBELLUM: TRANSMITTERS AND HISTOCHEMISTRY

318.1 GLUTAMATE AND ASPARTATE BINDING IN RAT CEREBELLUM: CHARACTERISTICS AND DISTRIBUTION. W. Sun* and N.S. Nadi* (SPON: R.J. Porter) Section on Psychogenetics, NIMH, Bethesda, MD 20205.

The cerebellar Purkinje cells are innervated by two excitatory inputs: the climbing fibers (CF) and the parallel fibers (PF). The transmitter released by PF is thought to be glutamate (glu) and that by the CF aspartate (asp). In this study we investigate: a) whether glu and asp receptors may be distinguished from each other, b) how these receptors are distributed in the cerebellum, c) how they respond to the lesioning of CF by 3-acetylptyridine (3-AP).

The binding assays were carried out on washed P_2 membranes, with ^{3}[H] asp and ^{3}[H] glu as ligands and unlabelled asp and glu as displacers. The incubation was in 50 mM tris buffer for 15 minutes at 37°C and was terminated by centrifugation at 4°C. Glu binding sites had a B_{max} of 270 pmol/mg protein, and asp a B_{max} of 85 pmol/mg protein. Glu and asp were equipotent in displacing each other. The K_d for glu and asp were 400 nMol, and 675 nMol respectively. The glutamate displacer quisqualate was ineffective against this site, whereas the DL-α aminoadipate was an effective displacer. Kainic acid had negligible affinity for this site. To determine the distribution of this binding within the cerebellum it was dissected at -20° C into the molecular, granular, white matter and nuclear layers. The distribution of glu binding in these layers was as follows: 625 $\pm$ 27 pmol/mg protein; 420 $\pm$ 39 pmol/mg protein, 150 $\pm$ 21 pmol/mg protein, and 272 $\pm$ 41 pmol/mg protein. The distribution of asp binding in the same layers was: 255 $\pm$ 25 pmol/mg protein; 150 $\pm$ 10 pmol/mg protein, 43 $\pm$ 19 pmol/mg protein and 190 $\pm$ 39 pmol/mg protein. The relatively high B_{max} of asp in the nuclei may be accounted for by the fact that the climbing fibers send collaterals into this area. The distribution of these receptors is in agreement with what is known about the anatomy and the physiology of PF and CF. The effects of 3-AP were studied in the cerebellum 2 days after a sing IP injection (65 mg/kg) of the drug. The B_{max} of asp increased in the molecular and nuclear layers but no significant difference was observed in the B_{max} for glu. These findings coupled with the above observations indicate that the transmitters released from CF and PF act at two distinguishable sites.

318.2 IMMUNOCYTOCHEMICAL DISTRIBUTION OF ASPARTATE AMINOTRANSFERASE AND GLUTAMINASE IN THE CEREBELLUM. K.K. Skaggs*, R.J. Wenthold and R.A. Altschuler (SPON. F. Siegel). Department of Neurophysiology, University of Wisconsin, Madison, Wisconsin, 53706 and NINCDS, NIH, Bethesda, Maryland, 20205.

We have suggested that aspartate aminotransferase (AAT) and glutaminase (GLNase) may be enriched in neurons which use glutamate or aspartate as neurotransmitters and may serve as immunocytochemical markers for these neurons. Studies on the cochlear nucleus, retina and hippocampus have shown these enzymes to be concentrated in neurons where several lines of evidence suggest a neurotransmitter role for glutamate or aspartate. In the present study, we have determined the immunocytochemical distribution of AAT and GLNase in the cerebellum of the rat and guinea pig. GABA is believed to be the neurotransmitter of cerebellar Purkinje, Golgi, basket and stellate cells. There is substantial evidence showing that glutamate or aspartate is the neurotransmitter of cerebellar granule cells.

Antibodies to cytoplasmic AAT were produced in rabbits as previously described (PNAS 78, 6553, 1981), and GLNase was purified from rat kidney and antibodies made in rabbits. Animals were perfused with 4% paraformaldehyde and the indirect immunofluorescence technique was used on cryostat sections while the PAP technique was used on cryostat, vibratome or paraffin sections. Primary antiserum was used at dilutions ranging 1/200 to 1/3000. Controls included normal rabbit serum and absorption controls in place of primary antiserum.

Antibodies to GLNase showed intense labeling of granule cells. However, a population of granule cells consistently remained unlabeled. Antibodies to AAT showed labeling of granule cells, stellate cells, and baskets surrounding Purkinje cells. As with GLNase antibodies, there appeared to be two populations of granule cells, one labeling intensely with AAT antibody, and the other labeling only lightly. The presence of AAT and GLNase in granule cells is consistent with the suspected neurotransmitter role of glutamate or aspartate in at least a population of these neurons. The presence of AAT in neurons which are thought to release GABA suggests this enzyme may also play a role in the production of GABA.

318.3 ANATOMICAL EVIDENCE FOR A CORTICAL "X" ZONE IN THE CEREBELLUM OF THE CAT. J. Voogd* (Spon: M. LaVail). Dept. of Anatomy, Leiden, The Netherlands and NASA-Ames Res. Ctr., Moffett Field, CA 94035

The longitudinal division of the cerebellum is based on myeloarchitectonic and experimental data on the efferent and afferent (climbing fiber) connections of the Purkinje (P) cells. P-cell fibers from longitudinal cortical zones en route to their central cerebellar nucleus, occupy parasagittal compartments in the cerebellar white matter. The same compartments contains the olivocerebellar (OC) fibers which terminate as climbing fibers on the P-cells of the corresponding zones. Compartments are separated from each other by narrow spaces which contain small, presumably afferent, fibers, but which lack the larger P-cell fibers.

Longitudinal cortical zones can be characterized by their corticonuclear and OC projections and by the size of their P-cells and the P-cell fibers of the corresponding compartment. Six main zones (A, B, C_1, C_2, C_3, D) could be distinguished in the cat and other mammalian species (Voogd, J. and Bigare, F. in The Inferior Olivary Nucleus, J. Courville et al., Eds, Raven Press, 1980). According to the electrophysiological investigations of Ekerot and Larson (Exp. Brain Res., 36:202, 1979) an additional "x" zone is present between the A and B zones of the anterior vermis in the cat. It receives branches from OC fibers which also terminate in the lateral half of the C_1 zone, presumably originating from the rostral half of the dorsal accessory olive (DAO).

The presence of an X zone could be confirmed in the cat. It is restricted to the dorsal part of the anterior lobe (lobules IV and V) and the simple lobule (VI). Injections of horseradish peroxidase in the lateral part of the fastigial nucleus and at its junction with the posterior interposed nucleus show that X projects to these nuclei and that its P-cells are significantly smaller than those of the A, B, C_1 and C_3 zones. Corresponding differences in size can be observed between the P-cell fibers of the X and the A, B, C_1 and C_3 compartments in semithin, toluidin blue stained sections. Apart from the X zone, also the C_2 zone consists of small P-cells with thin axons, which lie intercalated between the larger elements of the neighbouring zones. 3-H leucine autoradiography and axon degeneration of the OC projection do not support a projection of the DAO to the X zone, but favour its origin from the medial accessory olive, together with the projections to the A and C_2 zones. It can be concluded that X and C_2 have a number of anatomical features in common.

318.4 CEREBELLAR BANDS OF 5'-NUCLEOTIDASE IN THE MOUSE:LOCALIZATION OF THE ENZYME IN PURKINJE CELLS. Douglas T. Hess*, Dept. of Psychology, MIT, Cambridge, MA 02139 and A. Hess, I. Cassady*, I.Meadows* and P. J. Adamo*, Department of Anatomy, Rutgers Medical School, UMDNJ, Piscataway, NJ 08854.

The first evidence for a compartmentalization of the cytoarchitectonically uniform cerebellar cortex was provided by Scott (Nature, 200:793, 1963), who showed with histochemical methods that the enzyme 5'-nucleotidase was distributed in longitudinal bands in the molecular layer of the mouse cerebellum. A clear structural basis for the zonal distribution of cerebellar 5'-nucleotidase has not yet been provided. The present study indicates that the 5'-nucleotidase disposed in bands in the molecular layer of the mouse cerebellum is localized in Purkinje cells.

Cryostat sections from mutant mice lacking specific cerebellar cell types were stained for 5'-nucleotidase according to Scott (op. cit.). Sections from homozygous mutants were processed together with those from heterozygous littermates.

In all heterozygous mice, as in wild-type mice, 5'-nucleotidase was disposed in bands of dense staining in the cerebellar molecular layer alternating with bands of much lighter staining. In homozygous weaver (*wv/wv*) mice of 37 and 53 days of age, the cerebellum was severely atrophied due to a lack of granule cells and depletion of Bergmann glia. Nevertheless, a banded distribution of 5'-nucleotidase was still apparent, indicating that neither granule cells nor Bergmann glia are responsible for the bands. In homozygous nervous (*nr/nr*) mice of 50 and 52 days of age, cerebellar morphology was grossly normal, although Purkinje cells were greatly reduced in number. The molecular layer was lightly and uniformly stained for 5'-nucleotidase in the *nr/nr* cerebellum with no evidence of banding. Similarly, in homozygous Purkinje cell degeneration (*pcd/pcd*) mice of 45 days of age with advanced depletion of Purkinje cells, no banding of 5'-nucleotidase was evident. In *pcd/pcd* mice of 30 days of age with less complete depletion of Purkinje cells, a vestigial pattern of banding could be discerned over a limited extent of the posterior vermis.

These observations suggest strongly that the banded distribution of 5'-nucleotidase in the molecular layer of the mouse cerebellum reflects the localization of this enzyme in the dendrites of sets of Purkinje cells, spatially organized to form longitudinal compartments. This intrinsic chemoarechitecture may be related to the longitudinal zonation of the cerebellar cortex demonstrated by experimental studies of cerebellar afferent and efferent connectivity.

Experimental studies employing selective neurotoxins are in progress.

Acknowledgements: D. H. supported by NIH grant EY00126.

318.5 DEVELOPMENT OF THE ACETYLCHOLINESTERASE BAND PATTERN IN CAT CEREBELLUM. B.L. Brown and A.M. Graybiel, Dept. of Anatomy, Emory Univ. Sch. of Med., Atlanta, GA 30322 and Dept. of Psychology and Brain Science, M.I.T., Cambridge, MA 02139.

Longitudinal bands of high and low acetylcholinesterase (AChE) activity are visible in the molecular layer of adult cat cerebellum. The edges of certain of these bands mark the boundaries of Voogd's A and B connectional zones (Brown and Graybiel, 1983). The longitudinal organization of the cerebellum appears to develop early, as elongated clusters of neurons have been observed prenatally (Korneliussen, 1969). We have traced the development of AChE staining in relation to the cerebellar cell clusters in frontal sections, processed alternately for demonstration of AChE and Nissl substance, from fetuses at ages E25-E60, neonatal to 7½ week kittens, and adult cats.

By E25, AChE-positive patches are present superficially in the cerebellar primordium, but not in the ventricular proliferative zone. The AChE-rich areas correspond to cell-sparse areas in Nissl-stained sections. By E39 AChE is demonstrable in an interrupted lamina in the medial part of the cerebellum. Beneath this is an AChE-poor layer which in Nissl sections contains discontinuous plates of cortical neurons. Though the depth distribution of the AChE- and cell-rich zones is largely complementary, the discontinuities in cell and AChE distributions are in register, suggesting that these longitudinally oriented cell-dense and AChE-positive bands are forerunners of the adult zonal pattern.

The laminar complementarity of AChE and cortical cell clusters is lost in a narrow anteromedial zone at E39. By E43 the overlap extends farther laterally. It is as though for the first time neurons are allowed to invade the AChE-rich lamina. At E52 the edges of the AChE-rich lamina are less sharp and the AChE overlaps with the Purkinje cell layer, now distinguishable in parts of the vermis. The AChE bands are still sharply defined, but the gaps between cell clusters have almost disappeared. Thus, by E52, the longitudinal organization of the cerebellum is more evident in the AChE histochemistry than in the Nissl-stained material.

From about E52 to the 3rd-4th postnatal week, a Purkinje cell-associated staining (PCAS) of indeterminate cellular localization is prominent in the AChE sections, reaching its peak around the time of birth. At E57 the PCAS is mainly perisomatic; by E60 it extends to the proximal dendrites; by PO-P3 the full Purkinje cell profile shows the PCAS. A decline in PCAS follows, with few positive profiles remaining medially at P13, and none by P30. From P30 on, the AChE bands have the mature molecular-layer pattern. At all stages examined there is a pronounced medial-lateral gradient, with the vermis leading the hemispheres in development.

Supported by the Emory University Research Fund, NIH RR 5364, the Sloan Foundation, NASA NAG2-124, and NSF BNS-12125.

318.6 EFFECT OF SURGICAL LESIONS ON CHOLINE ACETYLTRANSFERASE ACTIVITY IN RAT CEREBELLAR NODULUS C.D. Ross, R.P. Hellendall*, D.A. Godfrey. Dept of Physiology, Oral Roberts University, Tulsa, OK 74171

In rat cerebellar vermis the highest activity of choline acetyltransferase (ChAT), the enzyme synthesizing acetylcholine, is in the granular layer of the ventral nodulus (lobule X, Larsell) (Ross et al., J. Histochem. Cytochem., in press). The effects of two types of surgical lesions on ChAT activity in the ventral nodulus were studied: (I) lesions made by placing an "L" shaped probe through the dorsal cerebellum and rotating it through the inferior peduncle (IP) on (A) the left side only and (B) on both sides; (II) lesions made by placing a 5 mm long razor blade knife through the dorsal cerebellum, the left vestibular nuclear complex and medial to the left cochlear nucleus. The following table summarizes data from over 700 samples microdissected from 18 rats:

ChAT activity (μmoles/kg dry wt/min): overall average ventral nodulus ± SEM (left side average/right side average)

	no. animals	days survival	granular layer	Purkinje cell layer	molecular layer
Control	4	-	327 ± 11 (323/330)	144 ± 18 (140/148)	32 ± 2 (32/32)
IA	4	7	342 ± 44 (338/346)	252 ± 73 (272/230)	57 ± 21 (66/48)
IB	3	7	130 ± 17 (134/138)	74 ± 35 (67/81)	13 ± 5 (15/11)
II	3	2	238 ± 22 (243/232)	110 ± 4 (83/138)	33 ± 5 (29/37)
II	2	7	137 (130/145)	62 (46/77)	15 (15/15)
II	2	34	92 (74/111)	62 (54/70)	13 (13/13)

Lesioning only the left IP (IA) did not reduce ChAT activity in the ventral nodulus, whereas lesioning the IP on both sides (IB) reduced activity in all layers by 50-60%. The knife lesion (II) on the left side produced by day 34 a 70% loss of ChAT activity in the granular layer and 60% losses in Purkinje cell and molecular layers. These losses are greater than would be expected from lesions placed on only one side of the cerebellum. About 65% of the ChAT activity in the ML of both lesion and control animals was in the inner third next to the PCL, about 25% in the middle third and 10% in the superficial third. The reduction in ChAT activity following lesions would be consistent with the interuption of cholinergic mossy and climbing fibers terminating in the GL and ML, respectively.

Supported by Oral Roberts University Intramural funds.

318.7 THE DISTRIBUTION AND DEVELOPMENT OF SEROTONIN IN THE OPOSSUM CEREBELLUM, J.S. King, R.H. Ho and G.A. Bishop, Department of Anatomy and Neuroscience Research Laboratory, The Ohio State University, Columbus, Ohio 43210.

The pattern of distribution and development of serotonin (5HT) in the opossum cerebellum was studied using transverse and sagittal sections processed by Sternberger's PAP technique. In the adult, 5HT axons and varicosities are present in all cerebellar lobules and deep nuclei, however the fiber density is not uniform. The densest distribution is in vermal lobule VIII and the dorsal folia of lobule IX. Moderate 5HT immunostaining is present in vermal lobules I, VI, VII, X and the ventral folia of IX, as well as lateral parts of lobules IV and V, lobus simplex, crus I and II, and the paraflocculus. Other lobules of the anterior lobe (II-V), the paramedian lobe and the flocculus contain sparse 5HT immunolabeling. Within the granule cell layer of lobules VIII and IX, immunoreactive elements are most prominent in a midsagittal band and, to a lesser degree, in two parasagittal bands. Beaded 5HT axons course through the deep and middle portions of the granule cell layer and give rise to a plexus at the border between the Purkinje cell and granule cell layers. Within this plexus axons extend long distances in the transverse and sagittal planes. Long beaded fibers oriented in the transverse plane of the folia also are present in the deep molecular layer. A few radial 5HT fibers ascend to the pial surface and give rise to very short tangential branches. In all three cortical layers, the varicosities measure from 0.5-1.5 μm and form boutons en passant. Of the four deep cerebellar nuclei, the medial and lateral receive the densest projection.

At birth, postnatal day (PD) 1, 5HT axons are present in medial parts of the rostral cerebellar anlage. By PD 9 a moderate plexus of fibers is evident along the midline, whereas lateral parts of the cerebellar plate exhibit sparse 5HT immunostaining. By PD 17, 5HT elements are distributed throughout the cerebellum, but are not related to clusters of Purkinje cells which are seen at this age. The lobular distribution pattern described in the adult is present by PD 45, however the density of 5HT within individual cortical laminae has not been achieved. These data suggest that: 1) 5HT fibers distribute differentially to the lobules of the opossum cerebellum; 2) unlike the rat (Takeuchi et al., Cell and Tiss. Res., 1982, 226:1) the opossum does not appear to have an extensive distribution of 5HT in the molecular layer; 3) 5HT afferents reach the opossum cerebellum early in development, prior to the arrival of mossy and climbing fibers (Morgan et al., Anat. Rec., 1983, 205:134). (Supported by NS-08798 and NS-17080. We thank Dr. R. Elde for the 5HT antibody).

318.8 THE ORIGIN OF SEROTONINERGIC AFFERENTS TO THE CEREBELLUM OF THE RAT. G.A. Bishop and R.H. Ho, Department of Anatomy and Neuroscience Research Laboratory, The Ohio State University, Columbus, Ohio, 43210.

The origin of serotoningeric (5HT) afferents to the cerebellum of the rat was studied using a technique which combines the retrograde transport of horseradish peroxidase (HRP) with Sternberger's PAP technique (Bowker et al., J. Histochem-Cytochem, 1982). Previously, speculations on the source of 5HT to the cerebellum have been based on indirect data in which the location of 5HT positive somata in medullary and pontine raphe and reticular nuclei was correlated with the location of neurons retrogradely labeled with HRP. However, these raphe and reticular nuclei also contain non-serotoninergic neurons. In order to determine which raphe and reticular neurons give rise to the 5HT projection large bilateral pressure injections of HRP were made in the cerebella of five adult Sprague Dawley rats. In two cases, the animals were pretreated with parygyline and tryptophan to enhance 5HT labeling. Three types of neurons could be identified in the rat brainstem: 1) cells containing only HRP reaction product; 2) cells which only show 5HT-like immunoreactivity; and 3) cells which contain both retrograde HRP and 5HT labeling. The first group of neurons are located in all precerebellar nuclei. In addition, retrogradely labeled neurons are located in the paramedian reticular nucleus, the n. raphe pallidus, n. raphe obscurus, n. reticularis gigantocellularis, n. reticularis tegmenti pontis, n. reticularis pontis oralis and the n. centralis superioris. Neurons demonstrating 5HT immunoreactivity are located in all areas previously described by Dahlstrom and Fuxe (Acta. Scand. Physiol., 1964) and labeled groups B1-B9. Double labeled neurons are located just dorsal and lateral to the rostral dorsal accessory olive in the n. reticularis gigantocellularis (NRG; group B1). These double labeled neurons extend rostral to the inferior olive reaching the level of the trapezoid body. Here, they occupy more medial parts of the ventral NRG and comprise the B3 group. Finally, a population of HRP-5HT positive neurons are located rostrally in the n. reticularis pontis oralis (group B5). A few double labeled neurons are also observed in the n. centralis superioris. Virtually no double labeled neurons are present in other raphe or reticular nuclei. In conclusion, this study has demonstrated that there is a fairly widespread distribution in the origin of medullary and pontine reticular afferents to the rat cerebellum. However, our data suggests that, the origin of serotonin to the rodent cerebellum is primarily located in the ventral and rostral aspects of the n. reticularis gigantocellularis and the n. reticularis pontis oralis. (Supported by NS 18028 and NS 17080; we thank Dr. R. Elde for the 5HT antibody).

318.9 ANALYSIS OF ELEVATED CEREBELLAR NOREPINEPHRINE LEVELS IN THE GENETICALLY DYSTONIC RAT: AN ANATOMICAL AND PHARMACOLOGICAL INVESTIGATION. T. W. McKeon and J. F. Lorden. Departments of Anatomy and Psychology, University of Alabama in Birmingham, Birmingham, AL 35294.

The dystonic (dt) rat is an autosomal recessive mutant that displays a behavioral syndrome characterized by torticollis, hyperflexion of the trunk, frequent falling to the side, and poor limb placement during ambulation. Routine light microscopy revealed no gross morphological lesions in either neural or non-neural tissues of dt rats. However, several neurochemical abnormalities, including significantly elevated resting levels of cerebellar norepinephrine (NE) have been found in dt rats in comparison with unaffected littermates. NE levels in other regions appeared unaltered. The elevated NE levels in the cerebellum of the dt rat may indicate either a hyperinnervation of the cerebellum or an abnormality in NE metabolism. The present study was undertaken to examine the pattern of noradrenergic innervation in the cerebellum and the response of this system to a pharmacological agent.

Light microscopic examination of cresyl violet stained sections of the cerebellum revealed no abnormalities in foliation or in the location of Purkinje cells, the cells that receive the major cerebellar noradrenergic input. Purkinje cells in dt rats displayed a prominent nucleolus and no obvious degenerative changes. Planimetric measurements of cell size made from camera lucida tracings indicated that the Purkinje cells of 20 days old dt rats were smaller than those of phenotypically normal littermates. The locus coeruleus innervation of the cerebellar cortex was examined in 20 day old dt and normal rats using the glyoxylic acid histochemical fluorescence technique. Normal and dystonic rats displayed similar patterns of NE innervation, but the presence of fluorescent fibers was more clearly evident in the dt rats than in the controls. Although this is not a quantitative technique, the increased fluorescence seen in the dt rat did not appear to be the consequence of an increased density of NE fibers, rather the fluorescent intensity of the fibers appeared to be increased in the dt rats.

In the pharmacological studies 20 day old dt and normal rats were treated with intraperitoneal injections of 0, .2, .4, or .6 mg/kg of reserpine and decapitated 18 h later. At all doses, dt rats demonstrated less depletion of cerebellar NE that did normal rats. Taken together, the anatomical and pharmacological observations suggest that the elevation in resting NE levels seen in the cerebellum of the dt rat may be due to a decreased release of NE. However, other explanations such as a more rapid replacement of released NE have not yet been ruled out. (Supported by NINCDS grant NS18062).

318.10 INCREASED GLUTAMIC ACID DECARBOXYLASE ACTIVITY IN THE DEEP CEREBELLAR NUCLEI OF THE GENETICALLY DYSTONIC RAT. G.A. Oltmans, J. Gordon and M. Beales. Dept. of Pharmacology, Chicago Medical School, North Chicago, IL 60064.

A mutant strain of Sprague-Dawley rats with motor characteristics similar to those of human torsion dystonia has been identified (Lorden et al., 1981, Soc. Neurosc. Abst. vol. 7). The syndrome includes a stiff gait with frequent falling, excessive circling, torticollis, self-clasping of forelimbs and hindlimbs, and hyperflexion of the trunk. Although light microscopy studies have failed to find any structural abnormalities in peripheral motor systems, muscle, or the CNS of the dystonic mutant, neurochemical studies have revealed differences between dystonic rats and their normal littermates in specific CNS structures. In this respect, cerebellar norepinephrine (NE) levels are significantly elevated (~+30%) in the dystonic mutant, while NE levels in the hippocampus and cortex do not differ from normal rats. The dopamine levels in the cortex and striatum also do not differ between normal and dystonic rats.

The finding of increased cerebellar NE levels suggests a dysfunction in adrenergically-mediated control of cerebellar Purkinje fibers which could, in turn, lead to other cerebellar abnormalities. Consequently, in the current study an analysis of glutamic acid decarboxylase (GAD) activity was made in specific cerebellar areas.

Studies were conducted in normal and dystonic animals 16 and 20 days old, ages when the dystonic syndrome is fully expressed. In 16 day old rats GAD activity was significantly increased in the deep cerebellar nuclei of the dystonic mutant (17.0±2.9 nmol/mg tissue-hr vs. 13.8±1.1, $P<.05$), while GAD activity in the cerebellar hemispheres (dystonic=9.8±2.7 normal=10.2±1.0) and vermis (dystonic=9.6±2.1; normal=9.1±1.4) was not different. Similar results were found in 20 day old rats (deep nuclei, dystonic=20.3±5.0, normal=15.4±2.2, $p<.05$; hemispheres, dystonic=7.4±0.8, normal=8.1±0.8; vermis, dystonic=5.9±0.4, normal=6.2±0.4).

The results indicate significantly increased GAD activity in the deep cerebellar nuclei of the dystonic mutant. The increased GAD activity may reflect a failure of the NE projections to the Purkinje cells to inhibit these cells. Alternatively, the GAD changes may be a secondary developmental consequence of the dystonia and not a result of the abnormal NE per se. An analysis of GAD activity in other brain regions (corpus striatum, globus pallidus) is under study. (Supported by NIH Grant NS-18062).

319.1 THE MATERNAL FOOD MANIPULATION EFFECTS ON PUPS'S CORTICOSTERONE* LEVEL AND THE MILK TRANSPORTATION OF CORTICOSTERONE: S. Gershon, M. Sakuma,* D. Caldwell,* C. Hofer.*

It is hypothesized that the maternal condition such as food intake may cause a change of plasma corticosterone concentration in pups at weaning time. To investigate this hypothesis, the weaning time was selected for measuring corticosterone levels because corticosterone has been established to appear somewhere between 18 and 25 days of age (Ader, 1969). The maternal factor was managed by feeding Pregnant Rat Diet (Bio Serve Co., #711-PR). Pregnant Group A dams were given the diet only during the third trimester while Group B dams were fed the same diet during the second and third trimesters. These dams were managed by Purina Lab Chow at all other times. Dams of controls were fed Purina Lab Chow throughout their pregnancy and lactation periods.

On day 23 postpartum 16 pups from groups A and B were compared to each other and to 16 control pups for corticosterone level. No difference was observed between group A progeny and the control subjects. However, group B pups produced significantly higher corticosterone levels when compared to both group A ($p < 0.05$) and control pups ($p < 0.05$). These results suggest that a longer duration of food change during dam pregnancy has a greater influence on corticosterone leveles in their progeny.

Because the one week food change elicited no observable effects, we compared corticosterone levels of dams with those of pups to examine possible milk transportation of corticosterone. Three pregnant rats were fed the aforementioned diet for one week during their third trimester. Although the corticosterone levels of dams measured immediately prior to the start of the third trimester (diet start), at end of third trimester (diet end) and end of lactation were not different; a random sample of 12 pups from the three dams revealed corticosterone levels one third lower than the dams when tested at birth. The corticosterone levels of 12 pups at day 23 (weaning time) were equal to that of dams' levels. Because day one pups were not receiving dams' milk, whereas day 23 pups were fed dams' milk. The coincidence of corticosterone levels at weaning time suggests the transportation of corticosterone through milk. This finding also supports the findings of Paye et al. (1977), Pealman et al. (1982) by proving the existence of corticosterone binding proteins in dams' milk. Morever, the low level of day one old pup corticosterone indicates no interference of CNS-pituitary adrenal axis at this early life.

319.2 ANDROGEN INDUCED PROLIFERATION IN THE LARYNX OF JUVENILE XENOPUS LAEVIS, D. Sassoon*, N. Segil*, and D. Kelley (SPON: R. Silver) Dept. of Biological Sciences, Columbia University, New York, N.Y. 10027:

We have examined the effects of androgen on the larynx, the vocal organ, of Xenopus laevis. The sex-specific vocal behaviors of the South African clawed frog are sensitive to circulating androgens (Wetzel and Kelley, Hormones and Behavior: in press; Hannigan and Kelley, this volume). We report here that testosterone induces a surge of proliferation in the larynx of sexually immature frogs (juveniles). This proliferative response may contribute to the marked sexual dimorphism in size and weight of the adult cell populations of laryngeal motor neurons (Hannigan and Kelley, Neurosci. absts.,7:1981).

Testosterone pellets were implanted into juveniles followed four days later by a single injection of tritiated thymidine. Animals were sacrificed twenty-four hours later and processed for autoradiography. In early and late post-metamorphic juveniles (1 to 9 months after metamorphosis) substantial label was noted over laryngeal tissue. The proliferative response was not sex-dependent, nor did it vary significantly with the age of the juveniles used. Studies with sexually mature males, females, and ovariectomized females reveal little or no label, suggesting that the onset of sexual maturity diminishes the ability of laryngeal tissue to proliferate in response to androgens. No proliferation was seen in response to the administration of estradiol, suggesting that this effect is mediated specifically by the action of androgens as opposed to an estrogenic metabolite.

In both juveniles and adult ovariectomized females, treatment with androgens causes an increase in laryngeal weight (Kelley, Absts. Int. Ethol. Congr. 1981). In testosterone treated juveniles, the perichondrial layer undergoes marked hyperplasia. In preliminary EM studies, this layer was seen to contain partially differentiated muscle. Satellite cells (Mauro, A., J. Biophys. Biochem. Cytol. 1961) are a prominent feature of normal laryngeal juvenile muscle. We are presently using EM autoradiography to identify which cell types are involved in the androgen response.

Supported by HD16741

319.3 ANDROGEN BINDING IN THE LARYNGEAL MUSCLE OF XENOPUS LAEVIS: SEX DIFFERENCES AND HORMONAL REGULATION. N. Segil*, L. Silverman*, D. Kelley and T. Rainbow(SPON: Don Hood). Dept. Bio.,Columbia University, NY, NY 10027 & Dept. Pharm., Univ. Pennsylvania, Med. Sc. Philadelphia, PA. 19104: This abstract reports the existence of different concentrationsof cytosolic androgen receptor in male and female laryngeal muscle of the South African clawed frog, Xenopus laevis. We also present evidence that the amount of androgen specific binding in the adult female laryngeal muscle can be increased by treatment with testosterone.

The experiments were carried out on adult frogs, gonadectomized one week prior to receptor assay. The cytosolic fraction from freshly dissected laryngeal muscle was incubated with 6nM tritiated methyltrienolone(R-1881), a synthetic androgen. Cytosol from thigh muscle was also assayed as an internal control on a non-sexually dimorphic muscle. Non-specific binding was measured by co-incubation with 100 fold excess of non-radioactive R-1881. Specificity was assayed by co-incubation with 100 nM testosterone(T), dihydrotestosterone(DHT), estradiol(E), progesterone(P), and corticosterone(B). Assays were duplicated in the presence of triamcinolone acetonide(TA), a progesterone analogue. Steroid bound to receptors was separated from free steroid on Sephadex LH20 columns. Results are expressed as femtomoles steroid specifically bound/mg protein(±S.E.M.). Laryngeal tissue from 5-8 frogs was pooled for each assay and assays on each experimental group(male, female, and androgen treated female) were repeated at least 4 times. Adult male laryngeal muscle contained a mean receptor concentration of 21.0fm/mg(±0.9). The specificity studies resulted in 74.6% competition with T, 80.2% with DHT, 42% with E, 15% with P, and 13% with B. No differences in competitions with or without TA were seen. The mean androgen specific receptor concentration in female laryngeal muscle was 6.3fm/mg(±1.1). To determine if sex differences in androgen binding are modulated by circulating androgens, gonadectomized females were treated for 4 weeks with 20mg testosterone propionate pellets which were removed one week prior to assay. The mean androgen specific binding in laryngeal muscle of androgen treated females was 19.5fm/mg(±2.4). Thigh muscle in both sexes contained 1-3fm/mg specifically bound androgen. This figure was unchanged in the T treated females.

Thus, androgen receptor levels in laryngeal muscle are 3.5 fold higher in males than in females and the level of receptor binding in females can be tripled by T treatment. These results indicate that laryngeal muscle is an androgen target and suggest that changing levels of circulating androgen could influence development of the sexual dimorphism observed by Hannigan and Kelley (Abst. Soc. Neurosci.,1981) in the adult larynx and laryngeal motor neurons.

Supported by Grant HD16741

319.4 ANDROGEN EFFECTS ON SEXUALLY DIMORPHIC VOCAL BEHAVIOR AND LARYNGEAL MOTOR NEURONS OF ADULT FEMALE XENOPUS LAEVIS. P. Hannigan*, and D. Kelley. (SPON: D. Gorlick). Dept. Biol. Sci., Columbia Univ. New York, NY 10027:

The South African clawed frog, Xenopus laevis, exhibits sex typical vocal behavior. These differences are reflected in the motor neurons which run the behavior, those of cranial nerve IX-X (Hannigan and Kelley, Abstr. Soc. Neurosci., 1981). Typically males have more large motor neurons and more motor neurons overall than females ($\bar{X}$#=Male:610, Female:379; Mann Whitney U-test, $p < .001$)($\bar{X}$% of motor neurons $> 320\mu^2$ =Male:36%, Female:10.5%). The results of the present study indicate that in sexually mature female frogs, circulating androgen influences the number and size of motor neurons in nucleus IX-X as well as certain parameters of the female-typical vocalization.

Seventeen ovariectomized females were treated with testosterone(T) or dihydrotestosterone(DHT) and 10 sham operated females were treated with cholesterol(C). Temporal characteristics of male and female vocalizations were analyzed by measuring the interclick interval(ICI, the click is the basic unit of both male and female vocalizations). Frequency characteristics of individual clicks were measured on a Fast Fourier Spectrum Analyzer. Androgen treatment of females decreased the ICI and induced high frequency components in the female vocalization. These androgen-induced vocalizations resembled, but were not identical to, male-typical mate calls.

Following behavioral testing, the brains of the experimental and control females were processed for cell counting (nissl-stain). Androgen and cholesterol treatment significantly reduced the number of motor neurons in nucleus IX-X to 50% of the normal female number (T-treated females:170, DHT-treated females:165, C-treated females:184)(Mann Whitney U-test, $p > .10$). Also the distribution of large to small cells in nucleus IX-X increased by 25% as a result of androgen treatment. Cholesterol had no effect on cell size in contrast to its effect on cell counts.

Although complete expression of male-typical CNS morphology and behavior was not induced in females treated with androgen, our results indicate that certain female-specific parameters of CNS morphology and behavior remain sensitive to androgen treatment in adulthood.

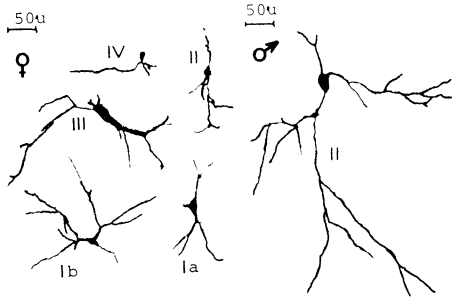
Supported by Grant HD16741

319.5 SEXUALLY DIMORPHIC NEURONS OF THE VOCAL PRODUCTION NUCLEUS IN XENOPUS LAEVIS: A QUANTITATIVE STUDY. D.B. Kelley and S.B. Fenstemaker. Dept. of Biol. Sci., Columbia Univ., N.Y.,N.Y. 10027 and Dept. of Psychol., Princeton Univ., Princeton, N.J. 08544

Sexual dimorphism underlies the sex specific vocal behaviors which are a prominent feature of the reproductive repetoire of the South African clawed frog. The vocal organ, the larynx, is 3 to 4 times larger in males than in females; laryngeal motor neurons are more numerous and are larger in somal size in males than in females (Hannigan and Kelley, Abstr. Soc. Neurosci.,1981). This study examined male and female laryngeal motor neurons for morphological sex differences.

The brains of 7 male and 5 female Xenopus laevis were impregnated using a Ramon Moliner modification of the Golgi-Cox technique. Camera lucida drawings of 176 female and 135 male neurons were analyzed. Ninety-six of these neurons fell into 3 distinct classes based on somal morphology: Types Ia and Ib (triangular somata), Type II (round or ovoid somata), and Type III (elongated somata). All four morphological categories were represented in similar percentages in both sexes. The mean number of primary dendrites within each class was not significantly different for males and females. Male type II neurons were significantly larger in somal diameter than female neurons and Type Ib and Type II male cells had longer cumulative mean lengths of primary dendritic segments. A Scholl analysis revealed that the dendritic branching patterns of male and female neurons were similar. However, at all orders of branching, dendrites of male neurons were longer than those of females. The dendritic trees of all classes of male laryngeal neurons are at least 1 order of magnitude longer than those of females, a very striking sex difference.

Supported by Grant HD16741



Representative neurons from each morphological class in females. A male Type II is shown at the same scale for comparison.

319.6 UTERINE POSITION AND CONDITIONED TASTE AVERSION. Anna M. Babine* & William P. Smotherman. Laboratory of Psychobiological Research, Department of Psychology, Oregon State University, Corvallis, OR.

Experiments have shown the existence of a sex difference in the acquisition and extinction of conditioned taste aversion (CTA), with males showing rapid acquisition and prolonged extinction compared to females. Female rats that develop in a uterine horn where caudal males are present exhibit masculinized reproductive behavior. Three experiments were designed to determine the influence of uterine position on the performance of female rats in a CTA paradigm. The offspring of Sprague-Dawley female rats and Long-Evans males were delivered by Cesarean section and toe-clipped to identify their uterine position. Three groups were formed based on uterine position: females with caudal male littermates (MF), females with no caudal male littermates (FF), and males (M) from a variety of uterine positions. As adults (65-79 days) the rats were tested for acquisition and extinction of a lithium chloride (LiCl)-induced CTA. In Experiment 1 the three groups were tested for acquisition of a CTA in the absence of exogenous hormonal manipulation. Experiment 2 compared extinction rates for the three groups, again in the absence of exogenous hormonal manipulation. The results of these two experiments confirmed a sexual dimorphism in rates of acquisition and extinction of a conditioned taste aversion, but failed to show a difference between MF and FF females. In Experiment 3, MF and FF females were given daily injections (.25 mg) of testosterone propionate (T) dissolved in sesame oil or pure sesame oil. A CTA was induced by i.p. injection of LiCl with controls receiving the volumetric equivalent of saline. Extinction rates for the various groups were compared. The results of this experiment indicate a differential sensitivity to exogenous T administration between MF and FF females. Rats in the MF group responded to administration of T with a pattern of prolonged extinction typical of males while FF rats failed to show an effect of T manipulation. These results indicate that the prenatal hormonal milieu (specifically T levels during the sensitive period) have an organizational effect on certain brain areas in the female rat. Circulating levels of T are insufficient to express these organizational effects, but exogenous administration of T activates these brain areas and results in a differential behavioral response in CTA.

WPS is supported by Grant NICH & HD 16102-02 and a grant from Oregon State University 30-262-0494.

319.7 ACCUMULATION OF STEROID IN THE SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA IN THE NEONATAL RAT HYPOTHALAMUS. J.N. Schoonmaker*, S.M. Breedlove, A.P. Arnold, R.A. Gorski. Lab. of Neuroendocrinology, Brain Res. Inst., UCLA Sch. of Med., Los Angeles, CA 90024.

A dramatic morphological sex difference in the rat hypothalamus, the sexually dimorphic nucleus of the preoptic area (SDN-POA), appears to be a morphological signature of gonadal steroid action on the brain. It is not known, however, whether the neurons of the SDN-POA are directly responsive to steroids during the critical period for sexual differentiation of the brain. The purpose of this study was to determine whether cells of the SDN-POA accumulate androgen and/or estrogen on day 2 of life, a time in development that this nucleus is known to be sensitive to exogenous steroids. Male and female pups were gonadectomized and adrenalectomized under cold anesthesia on the day of birth (day 1). On day 2, 2 pups of each sex were injected sc with ^{3}H-moxestrol (synthetic estrogen not bound by α-fetoprotein; 1.7 nmol/100g BW) or ^{3}H-methyltrienolone (synthetic non-aromatizable androgen;1.7 nmol/100g BW). After 2 hr, pups were killed, their brains removed and frozen in the deGroot angle onto a cryostat chuck, sectioned at 6 µm, and collected onto NTB-3 emulsion-coated slides. Autoradiographic slides were exposed for 4 and 7 weeks, developed, stained with thionin, and examined microscopically for steroid accumulation. Specifically labeled cells were identified as those cells with a silver grain density at least 5 times that of background (adjacent neuropil). There was no accumulation of ^{3}H-methyltrienolone in the region of the SDN-POA of male or female pups. In contrast, ^{3}H-moxestrol did label cells in the SDN-POA of both male and female pups. There was a gradient of decreasing estrogen accumulation from anterior to posterior within the SDN-POA (range: 22-50% labeled cells in anterior-SDN-POA; 0.8% labeled cells in posterior-SDN-POA). Interestingly, there was a high degree of estrogen accumulation in the cells of the medial preoptic nucleus surrounding the posterior portion of the SDN-POA (26-42% labeled cells). The capacity of SDN-POA cells to accumulate estrogen and not androgen on day 2 of life supports the notion that estrogen acts directly on these cells during sexual differentiation. Further, the anterior to posterior gradient of estrogen-labeled cells is consistent with the similar temporal gradient of neuronal formation and/or survival observed previously in relation to the final anterior-posterior position of cells within the SDN-POA. Whether these events are causally related remains to be determined. The unexpected but exciting observation of a halo of labeled cells surrounding the posterior portion of the SDN-POA leaves open the possibility of an indirect action of steroids on portions of the developing SDN-POA or the potential movement of steroid-responsive cells into the SDN-POA as it matures. NIH grants HD15021, HD1182, HD7228, and NSF grant BNS 80-06798.

319.8 SEX DIFFERENCES IN THE PREOPTIC AREA AND THE BED NUCLEUS OF THE STRIA TERMINALIS IN THE GUINEA PIG ARE NOT A FUNCTION OF THE ADULT HORMONAL ENVIRONMENT. M. Hines, A. Coquelin, F.C. Davis*, R.W. Goy and R.A. Gorski, Lab. of Neuroendocrinology, Brain Res. Inst., and Dept. of Anatomy, UCLA Sch. of Med., Los Angeles, CA 90024 and Wisconsin Regional Primate Res. Ctr., University of Wisconsin, Madison, WI. 53706

There is a marked morphological sex difference in the medial preoptic area (MPOA) of the rat brain. The volume of a darkly-staining portion of this region is several times larger in male than female rats (Gorski et al., Brain Res. 148:333, 1980). A striking sex difference also exists in the same region of the MPOA in the guinea pig brain (Hines et al., Biol. Reprod. 26 Suppl. 1:49A, 1982). We now report that 1) a morphological sex difference similar to that seen in the MPOA exists in another region of the guinea pig brain, the bed nucleus of the stria terminalis (BNST) and 2) the sex differences in the guinea pig MPOA and BNST are not a function of the adult hormonal environment. We analyzed brains of four groups of adult guinea pigs (Topeka stock): intact males, intact females, gonadectomized males and gonadectomized females. Each group included 5 or more animals. For the gonadectomized groups, surgeries were performed when animals were adult, and at least 2 weeks prior to sacrifice. Animals in all four groups were perfused with 10% formalin under Nembutal anesthesia and their brains were frozen-sectioned at 60 µm and stained with thionin. Sections were then projected at 43-fold magnification, and the boundaries of darkly-staining portions of the MPOA and BNST were traced by three investigators acting independently and without knowledge as to treatment group. Volume calculations were based on averages of these three drawings. The darkly-staining portion of the MPOA was found to be 4 to 5 times larger in male than in female guinea pigs, regardless of gonadal status. The BNST showed a similar, but less marked, sex difference (approximately 2-fold) that was also present in both intact and gonadectomized animals. These results indicate that dramatic morphological sex differences in the brain are not limited to the MPOA and are independent of the adult gonadal hormonal environment, at least in the guinea pig. These neural sex differences probably result from the action of gonadal steroids during early development. Both the MPOA and the BNST bind high levels of steroids during perinatal periods when hormones are known to influence behavior, gonadotropin regulation and the morphology of the rat MPOA. Morphological sex differences may be characteristic of areas that bind high levels of steroids at this time. Moreover, it is possible that these regions of the brain which are sexually dimorphic may comprise an anatomically and functionally interrelated neuronal system. Supported by NIH grants HD01182, RR00167, MH21313, HD5916, NS6594 and HD06160.

319.9 ESSENTIAL FATTY ACID DEFICIENCY DELAYS THE ONSET OF PUBERTY BY INHIBITION OF HYPOTHALAMIC AND OVARIAN FUNCTION. Sheryl S. Smith (White)* and S.R. Ojeda* (SPON: Carol A. Dudley). Department of Physiology, University of Texas Health Science Center at Dallas, Dallas, TX 75235.

The role of essential fatty acids (EFA) on sexual development was assessed in female rats born to dams fed a diet (Teklad #TD 77052) deficient in linoleic and linolenic acids begun a week before mating. The time of puberty and of first ovulation, as assessed by the age at vaginal opening and at first diestrus, was significantly delayed in EFA deficient (D) rats. The mechanisms underlying this delay appear to reside at both hypothalamic and ovarian levels. Experimental induction of preovulatory plasma estradiol (E_2) levels via implantation of E_2-containing Silastic capsules evoked an LH surge 30 h later in control juvenile rats, but not in D animals. This indicated immaturity of the hypothalamic component of E_2 positive feedback. Since prostaglandin E_2 (PGE_2) has been implicated in this process, the capacity of the hypothalamus to produce PGE_2 was examined next. Norepinephrine induced PGE_2 release from the median eminence of control rats in vitro, but it was much less effective in D rats. This was due to the EFA deficiency and not to reduced cyclooxygenase activity, as determined by incubation of hypothalamic homogenates with labeled arachidonic acid and subsequent HPLC separation of the products. A reduced gonadal synthesis of PGE_2 was also evident in ovaries from D rats which released significantly less PGE_2 than controls in response to hCG in vitro. Further signs of depressed ovarian function resulting from the EFA deficiency were a reduced gonadotropin receptor content and a blunted E_2 response to hCG in vitro. Altough prepubertal serum LH levels were unaltered, mean FSH titers were higher in D rats than in controls, probably as a result of a reduction in ovarian negative feedback signals. Conversely, the frequency of pulsatile FSH release was reduced in D rats, which may be due to the hypothalamic dysfunction. Pulsatile release of FSH was assessed at 30 min intervals throughout the day in conscious, freely moving 33 day old female rats implanted with indwelling jugular cannulae. These results suggest that delayed puberty resulting from an EFA deficiency may result from a delayed development of the hypothalamic capacity to respond to E_2 stimulation with a preovulatory surge of LH, as well as from a delay in ovarian maturation. Both of these insufficiencies may be due, at least in part, to reduced production of PGE_2 and, possibly, other arachidonic acid metabolites. (Supported by NIH Grant HD-09988).

319.10 SEQUENTIAL ESTRADIOL BENZOATE (EB) AND PROGESTERONE (P) ADMINISTRATION INDUCES A LUTEINIZING HORMONE (LH) SURGE IN GONADECTOMIZED MALE AND ANDROGEN-STERILIZED FEMALE GUINEA PIGS. W. Byne*, E. Terasawa, R. Bleier and R.W. Goy. Neuroscience Training Program, Dept. of Neurophysiology, Waisman Center and Wisconsin Regional Primate Research Center, University of Wisconsin, Madison, WI 53706.

We have previously reported that a single injection of EB (10 or 100 µg) elicits an LH surge in female but not male adult gonadectomized guinea pigs (Endocrinology, 104: 680, 1979). In the present study, the feedback effects of EB and P administration on LH release were examined in prenatally androgenized male and female guinea pigs as well as in control males and females. Timemated female guinea pigs received testosterone propionate (TP) or vehicle injections on days 28-65 of pregnancy. TP was administered daily at a dosage of 5 mg/day on days 28-37 and 1 mg/day on days 38-65 of gestation. Offspring were gonadectomized at approximately 120 days postnatally and ovaries were prepared for histological examination. After gonadectomy the animals received biweekly injections of EB (2.5 µg) and P (0.4 mg) for the purpose of behavioral testing. On the eighth week after gonadectomy the animals were fitted with indwelling jugular catheters and the effects of EB (10 µg) alone, and EB (1.5 µg) followed by P (1 mg 30 hr after EB) on LH release were examined. As described previously, blood samples were taken at 3 hr intervals and LH was measured by RIA. Results are: (1) The prenatal androgen treatment was effective in suppressing ovulation in the female guinea pigs as evidenced by the absence of corpora lutea in their ovaries (vaginal smears could not be obtained from TP-treated females due to the masculinization of their external genitalia). (2) EB induced negative feedback effects on LH release in all groups. (3) EB (10 µg) alone induced LH surges only in control females. (4) In contrast to EB alone, EB (1.5 µg) plus P (1 mg) induced LH surges in all groups of animals. In general, the amplitude of the EB-plus-P induced LH surge appeared to be attenuated in males and androgenized females compared to control females. Paradoxically, the LH surge in some androgenized males was augmented in duration but not diminished in amplitude relative to the surge in control females.

These data suggest that in the guinea pig prenatal androgen exposure suppresses the potential for spontaneous ovulation and masculinizes the neural substrate which mediates the positive feedback effect of estrogen on LH release. Prenatal androgen exposure, however, may not impair the mechanism which mediates the positive feedback effect of P on LH release, since P administration after a small priming dose priming dose of EB can induce an LH surge in male and androgen-sterilized female guinea pigs.

Supported by NIH grants RR-00167, NS16643, HD03352 and HD15433.

319.11 A DELAY IN THE EFFECT OF GONADECTOMY POSTNATALLY ON THE VOLUME OF SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA. L.P. Karaviti*, J. Schoonmaker*, J.E. Shryne*, and R.A. Gorski (SPON: E.H. Rubinstein). Laboratory of Neuroendocrinology, Brain Research Institute, and Department of Anatomy, UCLA School of Medicine, Los Angeles, CA 90024.

It has been shown that gonadectomy (GDX) shortly after birth reduces the volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) of the male when examined in adult life (Jacobson, et al., J. Neuroscience 1: 1142, 1981). In addition, the sex difference in SDN-POA volume has been shown to develop during the first 10 days of postnatal life (Jacobson, et al., J. Comp. Neurol. 193: 541, 1980); however, the time required for the effects of steroid action to be expressed has not yet been defined. In order to determine when gonadal hormones actually modify morphological differentiation, we examined the time course of the effect of GDX on day 1 of life on SDN-POA volume in gonadectomized and sham operated animals by analyzing brains of both sexes for SDN-POA volume on day 4 or day 8. Although there was a significant ($p < 0.01$) effect of age, sex and GDX on SDN-POA volume (3-way ANOVA), SDN-POA volume showed no effect of GDX by day 4 in both sexes. However, on day 8 the volume of the SDN-POA in gonadectomized males was only 57% when compared to that of the intact males of the same age. Moreover, SDN-POA volume of the gonadectomized males did not change over time. SDN-POA volume of gonadectomized males on day 4 = $7.17 \pm 0.89 mm^3 \times 10^{-3}$, while that of gonadectomized males on day 8 = $8.71 \pm 1.31 mm^3 \times 10^{-3}$. However, the volume of the sham operated males on day 8 was two-fold larger than that of the volume of the SDN-POA of sham operated males on day 4 ($15.38 \pm 2.38 mm^3 \times 10^{-3}$ and $7.60 \pm 1.03 mm^3 \times 10^{-3}$, respectively). Although these results confirm the influence of GDX on the development of the SDN-POA and suggest that exposure to gonadal steroids is required for the male during normal development, the results reveal an interesting delay in this effect. Since there was no significant effect of GDX by day 4, this implies that sometime between days 4 and 8 the absence of the stimulatory effect of gonadal hormones is expressed in a decrease in SDN-POA volume, apparently because of a failure of nuclear volume to increase. Whether the early development (that is, before day 4) of the SDN-POA is less dependent on steroids, whether the SDN-POA system is only primed by steroids during this early period, or whether the effects of steroid action merely require several days to be expressed morphologically remains to be determined.

Supported by USPHS NIH grant HD-01182.

319.12 THYROID HORMONE EFFECTS ON CYCLIC AMP METABOLISM IN CULTURED NEUROBLASTOMA CELLS. M. A. Walz and A. C. Howlett*. Dept. of Pharmacology, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

We have examined the effects of the thyroid hormone, triiodothyronine (T_3), on 3',5'-adenosine monophosphate (cAMP) metabolism in cultured neuroblastoma cells (N18TG2). Agents that increase cAMP levels in N18TG2 cells, e.g. prostaglandin E_1 (PGE_1), prostacyclin, and cyclic nucleotide phosphodiesterase (PDE) inhibitors, increase the degree of differentiation and decrease the rate of mitosis. In the developing intact animal, thyroid hormones first promote mitosis of neurones and later inhibit mitosis by promoting differentiation. We have tested the hypothesis that thyroid effects on developing neurones may result from alterations in cAMP metabolism. We have selected neuroblastoma cells in culture as a model system because of the ease of manipulation of the endocrine environment.

N18TG2 cells were grown in Bottenstein/Sato (Proc. Natl. Acad. Sci., 76:514, 1979) defined medium (without serum) for 48 hours with and without 10 nM T_3. The accumulation of cAMP in intact cells was increased by the thyroid treatment. Adenylate cyclase activity in an enriched plasma membrane fraction was increased. This increase was apparent for basal and PGE_1-, secretin-, fluoride-, guanosine 5'-(β-γ-imino)-triphosphate- and forskolin-stimulated activities, suggesting an alteration in catalytic activity rather than an increased population of prostaglandin or secretin receptors. Carbachol and norepinephrine inhibitions of adenylate cyclase were unaltered by T_3 treatment. High affinity cAMP PDE in the plasma membrane fraction from T_3-treated cells was reduced compared to controls, but low affinity (Ca^{+2}/calmodulin dependent) PDE activity was unchanged.

Dose dependence of T_3 effects was within two orders of magnitude. At 1 nM T_3, cAMP metabolism differed only minimally from control, and at 100 nM T_3, cell division was totally depressed. In summary, these results suggest that thyroid hormone effects on neuronal growth may be due in part to alterations in cyclic nucleotide metabolism.

Supported by NIH grant NS16513.

319.13 IMMUNOHISTOCHEMICAL EVIDENCE FOR A SEXUAL DIMORPHISM IN THE DISTRIBUTION OF DOPAMINERGIC CELLS AND FIBERS IN A PERIVENTRICULAR PREOPTIC NUCLEUS INVOLVED IN THE CONTROL OF GONADOTROPHIN RELEASE. R.B. Simerly*, L.W. Swanson and R.A. Gorski. Laboratory of Neuroendocrinology, Brain Research Institute and Department of Anatomy, UCLA School of Medicine, Los Angeles, CA 90024 and the Salk Institute, La Jolla, CA 92112.

A small cluster of cells at the rostral extreme of the third ventricle, designated here the anteroventral periventricular nucleus (AVPv), has been proposed to be involved in the control of gonadotrophin release (Wiegand et al., Neuroendo., 31: 147, 1980). These authors have reported that lesions of this nucleus block spontaneous ovulation, induce persistent vaginal estrous and abolish the progesterone-induced surge of luteinizing hormone in female rats. To investigate the possible relationship of monoaminergic neurotransmitter systems to the AVPv the distributions of biogenic amine immunoreactive elements within the nucleus were evaluated and a comparison made between males and females. Sections from adult male and female Sprague-Dawley rats were processed for immunofluorescence using antisera directed against tyrosine hydroxylase (TH), dopamine β-Hydroxylase (DBH) or serotonin and subsequently counterstained with the fluorescent Nissl stain ethidium bromide (Sawchenko and Swanson, Brain Res., 210, 31, 1981; Schmued et al., J. Histochem. Cytochem., 30: 123, 1982). The stage in the estrous cycle of the females was determined by vaginal smear on the day of sacrifice. In both sexes, very few serotonin immunoreactive fibers were seen within the borders of the AVPv, in contrast to the relatively high serotonin fiber density of the surrounding region. The most important finding of this study was a striking sexual dimorphism in the distribution of TH immunoreactive neuronal elements within the AVPv. In the female, the nucleus contained many TH stained fibers and perikarya, while very few TH immunoreactive elements were observed in the AVPv of males. A low to moderate density of DBH immunoreactive fibers and no DBH stained perikarya were seen in the nucleus. A clear sex difference was not found in the density of DBH immunoreactive fibers in the AVPv indicating that the sexual dimorphism in TH immunoreactivity in this nucleus is due to a greater density of both dopamine fibers and cell bodies in the female. These results taken together with the functional studies of Wiegand et al., indicate that dopamine may be involved in the control of a specific sexually dimorphic function: the preovulatory release of gonadotrophins.

We wish to thank Drs. K. Helle, T. Joh and H. Steinbusch for generous supplies of antisera.

Supported by NIH grants HD01182, HD7728 and NS16686.

319.14 MODULATION OF DENDRITIC MORPHOLOGY IN THE NEONATAL RAT MEDIAL PREOPTIC NUCLEUS FOLLOWING PERINATAL TESTOSTERONE ADMINISTRATION. M.E. Dato*, P.E. Meyers* and J.H. Gordon. Dept. of Anatomy and Dept. of Pharmacology, University of Health Sciences/The Chicago Medical School, North Chicago, IL 60064.

Using a modified Golgi-Cox technique, dendritic fields from the sexually dimorphic dorsomedial preoptic area were analyzed in 6 day old rat pups. It has been shown that this region can be influenced by gonadal hormones during the critical period for sexual differentiation of the brain. This study shows the effects of such hormone treatments on dendritic patterns are expressed at the time of exposure the hormone.

Female Sprague-Dawley pups were injected with 30,100 and 300 µg of testosterone propionate in sesame oil on day 1 (day of birth). Controls were injected with oil only. On day six pups were sacrificed by decapitation, brains removed and immediately placed in the Golgi solution. Brains were trimmed to $(4mm)^3$, embedded in parlodion and sectioned at 120 µm. Neurons were viewed at 400x and camera lucida drawings made. Cells were analyzed using an acetate overlay containing concentric circles of 21 µm (equivalent) diameters divided into octants centered on the cell soma. Octants were numered from 1 to 8 clockwise starting at "12 o'clock". Data were taken as ring crossings per octant. For this preliminary analysis, crossings per octant are expressed as percentages of total crossings.

	OCTANT (crossings/total crossings) x 100							
TREATMENT	1	2	3	4	5	6	7	8
30 µg TP* (48 cells)	12.7	9.9	9.1	10.8	21.8	12.0	11.0	9.3
100 µg TP (48 cells)	13.5	9.8	9.9	9.8	20.7	16.1	10.8	9.3
300 µg TP (46 cells)	21.0	10.1	3.7	10.5	23.1	15.9	6.6	8.8
Oil (55 cells)	8.1	24.2	19.3	3.5	5.2	14.3	15.2	10.1

*TP= Testosterone Propionate

Each of the treatment groups was found to differ significantly from the control (oil) group using a χ^2 analysis ($P<0.01$). These results indicate that perinatal testosterone treatment enhances dendritic growth in octants 1 & 5, thus giving a dorsolateral to ventromedial orientation. In contrast the treatment appears to lessen the lateral growth of dendrites (i.e. octants 2 & 3).

319.15 DENDRITIC ARBORIZATION OF NEURONS IN THE MEDIAL PREOPTIC NUCLEUS OF THE ADULT RAT. P. E. Meyers* and J. H. Gordon (SPON: J.M. Krueger). Department of Biological Chemistry and Structure and Department of Pharmacology, University of Health Sciences/The Chicago Medical School, North Chicago, IL 60064

In view of recent interest in the occurence of sexual dimorphism in the medial preoptic nucleus, we felt a need for a complete description of neuronal morphology as it occurs throughout the nucleus. In this study a modified Golgi-Cox preparation of adult rat brains (350 gm. Sprague-Dawley) was utilized. Serial sections were cut at 120 um and mounted sequentially on glass slides. Neurons observed to be reasonably complete were drawn from the entire medial preoptic nucleus in its entire anterior and posterior extent. Some neurons from adjacent medial preoptic area were also drawn for additional comparisons.

In general, two cell populations can be described. More ventrally and within the medial preoptic nucleus, relatively large cells can be found. These cells uniformly have ventrally situated dendrites. Each of these cells has one or two other dendrites that can extend in any direction from medial to dorsolateral. More dorsally in the medial preoptic area, and more caudally as well, a population of smaller cells can be found. The dendrites of these cells orient in a horizontal direction with one primary dendrite extending medially and another laterally. In current Golgi-Cox preparations these cells decrease markedly in frequency as more frontal sections of the medial preoptic area are examined.

The dendritic orientation of both cell types can be related to fiber systems coursing through the area. The dendrites of cells within the medical preoptic nucleus are consistently oriented perpendicular to fibers of the diagonal band. On the other hand, the dendrites of the cells located dorsally and caudally within the medial preoptic area are oriented perpendicular to fibers of the stria terminalis (thus confirming the observations of Fields and Sherlock; Golgi Centennial Symposium, Ed. M. Santini, Raven Press, N.Y., 1975).

319.16 DIFFERENTIATION OF SEXUAL AND AGGRESSIVE BEHAVIOR IN HAMSTERS BY ESTROGEN OR NON-AROMATIZABLE ANDROGENS. Eric T. Pleim* and Joseph F. DeBold (SPON: J.A. Politch). Dept. of Psychology, Tufts Univ., Medford, MA 02155.

Androgens present during perinatal sensitive period(s) determine the extent of masculinization and defeminization of a number of behaviors in rodents. Normally these developmental processes in male animals are mediated by testosterone, which is thought to exert its effects after conversion to estradiol (E) and/or dihydrotestosterone (DHT). We chose to compare the neonatal effects of E, DHT, and a synthetic androgen, R1881, on adult sexual and aggressive behaviors in hamsters.

Hamsters were injected with 0.5ug E, 250ug DHTP, 100ug R1881, or the oil vehicle 24 hours after birth. They were weaned and group housed by sex at 25-30 days. At 50-60 days they were singly housed and a week later tested for aggressive behavior with a male in a neutral arena. Next the animals were gonadectomized and tested for female sexual behavior (after 10ug EB + 500ug P treatment). They were then tested twice for male sexual behavior during two weeks of treatment with 500ug DHT, or 1 ug E and then after both hormones combined. After cessation of hormone treatments all animals were again tested for aggression.

All three neonatal hormone treatments, at least at the doses tested, only weakly defeminized females. However, all three neonatal hormone treatments did masculinize the females, although females treated with E showed the highest levels of male sexual behavior. Male behavior was not specific to the activating hormone used after gonadectomy; E and DHT were equally effective in adulthood. Only neonatal E treatment induced higher levels of male sexual behavior than vehicle treatment in males, and these males showed many more intromissions than the other groups. Control, R1881, and E males were equally defeminized. Interestingly, early DHTP apparently inhibited the defeminizing effects of the males' own gonadal steroids, probably through a negative feedback mechanism. However, this blockade of endogenous steroid action did not inhibit masculinization as measured by male sexual or aggressive behavior. In both sexes, a higher percentage of DHTP animals exhibited attacks in the aggression tests than any other group.

These results demonstrate that non-aromatizable androgens can cause changes in sexual differentiation, although they are less potent than estradiol. In addition, the neonatal effects of estrogens and androgens on masculinization do not result in changes in sensitivity that are specific to the hormone present during the sensitive period. Hamsters treated with estrogens (or androgens) neonatally show increased sensitivity to both androgens and estrogens for induction of male sexual behavior.

319.17 HYPOTHALAMIC LHRH CONTENT IN YOUNG AND MIDDLE AGED FEMALE RATS FOLLOWING OVARIECTOMY AND ESTROGEN-PROGESTERONE REPLACEMENT, Beverly S. Rubin*, Karen Elkind-Hirsch, and Robert S. Bridges. Dept. of Anatomy, and Lab. of Human Reproduction and Reproductive Biology, Harvard Medical School, Boston, MA 02115.

During middle age, female rats typically exhibit irregular estrous cycles and many subsequently enter a state of constant estrus (CE), a condition characterized by well developed follicles in the ovary, a lack of corpora lutea, and an absence of LH surges and ovulation. Deficits within the hypothalamic LHRH system may be responsible, in part, for this anovulatory condition since infusions of LHRH will induce LH surges in CE females. Whereas the attenuated LH surge elicited in older ovariectomized females in response to ovarian hormone administration has been well characterized, little information is available regarding age-related changes that may occur at the hypothalamic level involving either the synthesis and/or release of LHRH. In the present study, measurements of hypothalamic LHRH content in young and aging females were compared following administration of ovarian steroids. Young cycling rats (3-4 months) and middle aged CE females (10-15 months) were ovariectomized, and three weeks later they were injected with estradiol benzoate (4µg/100 gm BW) followed 48 hours later by an injection of progesterone (0.8mg/100gm BW). Females were sacrificed either just prior to progesterone (P) administration at 1000 hours or at 2, 4, or 6 hours after injection of the hormone. Brains were removed and the medial basal hypothalamus (MBH) and preoptic area were dissected and extracted for determination of LHRH content. Blood was collected for measurement of serum gonadotropins. Tissue LHRH content was assayed with LHRH antiserum CRR11B73 (supplied by Drs. Chen and Ramirez). The MBH-LHRH content of middle aged females differed significantly from the content measured in young females ($p<0.001$). At all time points, LHRH levels in the MBH of the aging females were higher ($\bar{X}$ =3.4ng/fragment) than those measured in the young controls ($\bar{X}$ =2.4ng/fragment); however, the LH surge was markedly attenuated in the middle aged females. Peak levels of serum LH in the older animals reached only 29% of the peak values determined for the young animals. These data suggest that the aging female has the ability to synthesize substantial amounts of LHRH, and that the age related cessation of estrous cyclicity may result, in part, from some impairment in the release of this hypothalamic peptide.

(Supported by NRSA HD 06282 from NIH awarded to BSR).

DEVELOPMENT AND PLASTICITY: BIOCHEMICAL AND PHARMACOLOGICAL CORRELATES II

320.1 ELEMENTS OF THE CHOLINERGIC SYSTEM ARE ASSOCIATED WITH THE MYOTOME OF THE RABBIT, V. M. Tennyson, L. T. Kremzner*, Z. Grubic*, and H. W. Chang*. Depts. of Anatomy & Cell Biology, Pathology (Neuropathology) and Neurology, Columbia University, College of Physicians & Surgeons, New York, N.Y., 10032.

We previously found acetylcholinesterase activity in the mononucleated myoblasts of the myotome of the rabbit at embryonic day 9 (E 9). Activity increased by E 13, the day before the myoblasts migrated away from the myotome to form multinucleated myotubes (Tennyson et al, JCB 51:703, 1971). Radioautography using [^{125}I]α-bungarotoxin at E 13 labeled the myotome, thus, the nicotinic receptor was also present (Grubic et al, Anat Rec 205:70A, 1983). We now report that choline acetyltransferase activity (ChAT) can be measured as early as E 10, which is before the spinal nerve has formed. To determine whether ChAT activity is confined to the nervous system or is also present in other tissues, we dissected the axial structures of eviscerated embryos into different samples. ChAT activities for the samples expressed as nMoles/hr/mg protein were as follows: nervous system-notochord = 9, 25.4; rows of myotomes separated from the previous sample = 14.2, 36.3. As a reference, the adult rat caudate-putamen = 45. At E 13, ChAT activity was present in the notochord, a neural tube-dorsal root ganglia preparation, as well as in rows of myotomes separated from the previous specimen. In an attempt to be sure that spinal nerves were not contaminating the rows of myotomes, single myotomes were dissected with as little adherent mesenchyme as possible and free of any visible nerves. ChAT activities for these specimens were 5.2, 6.7, and 11, whereas the corresponding nervous system-notochord specimens were 17.9, 13.1, and 13.3.

We noted in our toxin binding studies, cited above, that the "C"-shape curvature of the specimen, which contained neural tube, dorsal root ganglia, and myotomes was more pronounced after incubation in toxin. We documented this change by photographing this preparation before and after incubation. An increase in the curvature was apparent after a 5 min incubation and was marked after 4 hrs. We obtained similar results after incubating another preparation in carbamoylcholine, an analogue of acetylcholine. The myoblasts at E 13 have myofilaments, but only some of them are organized into sarcomeres. We have not found well defined axonal boutons until E 16. Nevertheless, our results suggest that mononucleated myoblasts of the myotome have elements that may permit them to participate in changing the shape of the embryo during development.

Supported by NS-11766, NS-13744, and the Muscular Dystrophy Associations of America.

320.2 THYROXINE EFFECTS ON THE DEVELOPMENT OF RAT CEREBELLAR CELLS IN CULTURE. Anne Messer, Paul Maskin*, and Gary L. Snodgrass. Ctr. For Labs and Research, N.Y. State Dept. of Health, Albany, N.Y. 12201.

The effects of hypo- and hyperthyroidism on the development of the rat cerebellum have been studied extensively. Thyroxine appears to induce early proliferation in the external granular layer, reduce the total amount of such proliferation, and induce premature maturation of the Purkinje cells. Cells in the external granular layer might either be affected directly by the hormone, or indirectly via precocious Purkinje cells. We have undertaken to study this question using serum-free monolayer cultures which lack Purkinje cells, but appear to contain the other major cerebellar cell classes.

Cells from 5-day-old rat cerebella were dissociated with trypsin, and plated on poly-lysine coated coverslips in Dulbecco's Modified Eagle's Medium (DMEM) plus 10% horse serum. After 6-24 hrs., the coverslips were inverted, and the medium changed to DMEM plus the Bottenstein and Sato serum-free hormone supplement as previously described (Messer et al, Cell. Mol. Biol 1,99-114,1981). Thyroxine (T4) or triiodothyronine (T3; the biologically active derivative of T4) were added at various times and concentrations.

Thymidine incorporation at 1,2,3, or 4 days in vitro after treatment of cultures with 20, 50,500, or 3000ng/ ml T3 or T4, started at one day after plating, yielded significant increases only at the highest concentrations of T3, labelled at 2 days. Extensive examination of autoradiograms showed that only non-neuronal cells had incorporated the nuclear label in any of the experiments. Increases in total protein /culture (measured after cultures had had three weeks to mature) were observed in addition at the more physiological concentrations of 50 and 500 ng T3/ml. There was also a significant decrease in the specific high-affinity uptake of GABA in the presence of the glial uptake inhibitor B-alanine, and a decrease in the specific activity of glutamic acid decarboxylase in treated cultures, apparently due to the increased protein contribution by non-neuronal cells.

We therefore conclude that T3 does not have a direct effect on the proliferation of neurons derived from the external granular layer, although it does seem to affect some non-neuronal cells. It also does not seem to enhance differentiated GABA functions (uptake or synthesis) beyond the levels achieved using insulin, progesterone, putrescine, selenium and transferrin.

Supported by NIH grant NS17633.

320.3 EFFECT OF DEXAMETHASONE ON THE CEREBRAL CORTICAL DEVELOPMENT OF FETAL RHESUS MONKEY. H. Uno*, C. S. Thieme*, J. W. Kemnitz and P. M. Farrell*. Wisconsin Regional Primate Research Center, Univ. of Wisconsin, Madison, WI 53715.

Administration of corticosteroids in large doses to newborn animals impairs brain growth and delays cortical dendritic branching. In this study, we observed cytoarchitectural changes in the cerebral cortex of fetuses that received dexamethasone prenatally. Pregnant rhesus monkeys were injected intramuscularly with 0.5, 5, 7.5, 10, or 15 mg/kg of dexamethasone at 132 days of gestation. Control animals received vehicle alone. At 72 hours following dexamethasone administration, fetuses were delivered by caesarean section. Brains were removed and fixed with 10% buffered formalin. Sections were cut from the following cerebral areas: frontal (area 9), precentral (area 4), postcentral (area 3), temporal (area 22), occipital (area 17), and hippocampal (area 27) gyri. Paraffin sections (10 μ thick) were stained with cresyl violet, silver impregnation, and luxol fast blue.

In the brains of 135-day fetuses (term of rhesus monkey = 165 ± 5 days), the cortical neurons were fairly well developed and the structure of layers I to VI could be recognized. Generally, the brains of animals treated with dexamethasone had fewer pyramidal cells, particularly in layer III of agranular and frontal cortical areas, compared with the brains of controls. Reduction of neurons in these cortical layers was more pronounced in brains from the higher dose groups; the number of pyramidal cells in layer III of the precentral area was 1545 ± 35/mm^2 in controls, 1297 ± 11 in 0.5 mg, and 1038 ± 19 in 15 mg dexamethasone-treated groups. In the cortex of animals from the 15 mg group, the pyramidal cells in layers III and IV of the precentral area showed shrinkage of perikarya with condensed cytoplasm. In the sensory cortices (postcentral and visual), the depth of the entire cortical gray (layers I to VI) was reduced in dexamethasone-treated animals compared with that of controls. The number of granular cells in layer IV of the postcentral gyrus (area 3) was markedly reduced in dexamethasone-treated animals: 6106 ± 71/mm^2 in controls, 4369 ± 145 in 0.5 mg, and 3979 ± 190 in 15 mg groups. Reduction of the cortical neurons was less prominent in the frontal, temporal, hippocampal, and visual cortices compared with that of the pre- and postcentral areas. The results suggest that prenatal treatment with corticosteroid can impair cerebral cortical development and can induce cytoarchitectural alterations. In this study, the degree of change was dose-dependent, with the neurons in the somatic motor and sensory cortices of prenatal rhesus monkeys appearing to be especially sensitive.

This work was supported by NIH Grants RR00167 and HL27358.

320.4 POSTNATAL CHANGES IN PROTEINS OF MOUSE DORSAL ROOT GANGLIA. L. J. Fisher, L. S. Stodieck*, J. A. Beel* and M. W. Luttges. Department of Aerospace Engineering Sciences, University of Colorado, Boulder, CO 80309.

Dorsal root ganglia provide the protein constituents for neural fibers predominantly in the peripheral nervous system. Only from the transition zone to spinal projection sites are these fibers myelinated with central nervous system oligodendrocytes. The ganglia, themselves, are immersed in a cerebrospinal fluid environment shared by the spinal cord. It is known that postnatal development in spinal cord differs considerably from that of peripheral nerve. Studies of the postnatal change in protein constituents of dorsal root ganglia should provide important insight into the processes which control postnatal development of neural tissue. The dorsal root ganglia of L_4-L_6 of mice ranging from 4-93 days of age were dissected from surrounding nerve tissues. The ganglia were weighed and then pulse labeled in vitro using ^{3}H-Lysine. Some of the labeled ganglia were subsequently prepared for SDS-PAGE separation of proteins. Acid soluble and insoluble radioactivity recovered from the remaining pulse labeled ganglia provided estimates of free amino acid pools and protein synthesis activity, respectively. The electrophoretically resolved proteins showed the developmentally-dependent alterations in relative protein concentrations of the dorsal root ganglia. From 4-93 days postnatally, the ganglia increased in wet weight three-fold from 95 to 329 μg per ganglion. Total protein recovery was approximately 6 to 21 μg per ganglion across the same time period. Thus, the ratio of wet weight to protein remained constant through the postnatal periods tested. Most of these gains were complete by the third or fourth postnatal week. The protein alterations corroborate this time course. Myelin protein accumulations are initiated at one week postnatally and are completed within the next two weeks. Nuclear histones show decreased prominence during these periods. Accumulations in relative amounts of tubulin, actin and neurofilament proteins appear most prominent at two weeks and persist for two additional weeks. Pulse labeling studies indicated that the uptake of amino acid and the incorporation into protein occur at uniformly high levels regardless of postnatal period. The postnatal development pattern of dorsal root ganglia suggests that some elements of this structure exhibit maturation rates similar to spinal tissues while others exhibit rates more like those shared by peripheral nerves. Nevertheless, Schwann-cell myelin proteins accumulate later in development at the ganglion level than in the peripheral nerves. This difference may reflect variations in control processes underlying central and peripheral neural development.

320.5 POSTNATAL CHANGES IN THE COMPOSITION OF MOUSE SPINAL CORD PROTEINS. L. S. Stodieck* and M. W. Luttges. Department of Aerospace Engineering Sciences, University of Colorado, Boulder, CO 80309.

Differences in the protein composition of neural tissues have been related to a wide range of normal and abnormal structures and functions. Major differences exist between proteins of peripheral nerve and brain tissue. In addition, changes in proteins during postnatal development are observed. Although the myelin proteins of spinal cord have been studied, there exists no comprehensive characterization of spinal cord proteins during postnatal development. The characterizations reported here were obtained by SDS-PAGE separations using 8.75-20.0% linear-exponential gels. Spinal cord tissues were obtained from mice 1-80 days old. Protein quantifications were completed using microdensitometric analysis of Coomasie Blue stained gels. More than 50 discrete bands were identified following separation of 30 μg samples of spinal cord protein. Of these, 40% exhibited increased relative concentrations during postnatal development. The remaining 60% of the bands were equally divided among those showing relative decreases or constancy of protein concentration during development. Meylin proteins (Wolfgram, proteolipid, DM-20 and basic proteins) appeared on postnatal day 8 and exhibited rapidly increased concentrations. Other myelin proteins did not accumulate at the same rates postnatally. Nuclear histones exhibited rapid decreases in relative concentration reflective of the appearance of myelin proteins. Similar decreases were observed for cytoskeletal proteins such as actin and tubulin. In contrast, neurofilament proteins showed slight increases in relative concentrations during spinal cord development. Myelin proteins appear earlier in peripheral nerve, at the same time in dorsal root ganglia and later in brain, postnatally. Cytoskeletal and neurofilament proteins also exhibit different patterns of relative accumulation in different neural tissues during development. Thus, the same species of proteins in neural tissue show accumulations specific for both species and locale. Such differences may be crucial to the functional and structural differences between these tissues. These developmental differences may provide a clue to important clinical distinctions such as neural regeneration.

320.6 NERVE GROWTH FACTOR RECEPTOR-RELATED PHOSPHORYLATION OF CYTOSKELETAL PROTEIN IN PC12 CELLS. Gary Landreth. Dept. of Anatomy Medical University of South Carolina, Charleston, SC 29425.

Nerve growth factor (NGF) acts on a clonal line of rat pheochromocytoma cells (PC12) producing a variety of changes associated with neuronal differentiation. The initial event in this interaction is the binding of NGF to specific cell surface receptors. NGF first binds to a low affinity receptor, followed by a conversion of a proportion of the receptors to a high affinity state. Coincident with the increase in receptor affinity is a change in the position or conformation of the hormone-receptor complex in the membrane and attachment to the cytoskeleton. It follows that PC12 cytoskeletons can be prepared which possess high affinity NGF receptors by pretreating the cells with NGF prior to detergent treatment, while the receptors on untreated cells will be removed with membrane solubilization. Using this preparation it was possible to detect in situ, receptor-related protein kinase activity.

PC12 cells were incubated in saline with or without NGF at 37°. The cells were centrifuged and the pellet resuspended in 25mM HEPES, pH 7.4 containing 0.15% Triton. After 2 min. at 4° the suspension was centrifuged and the pellet resuspended in the same buffer without detergent. The phosphorylation reaction was initiated by addition of γ-^{32}P-ATP or γ-^{35}S-ATP and carried out at 4° for 10 min. Incubation of the cells with NGF (0.38nM) resulted in the stimulation of labeling of a 250 kdalton protein. The NGF-stimulated labeling was dependent on Mn^{++}; Mg^{++} was ineffective. Similar results were obtained using either γ-^{32}P-ATP or γ-^{35}S-ATP.

Maximal stimulation of 250 kdalton labeling was observed within 5 min and declined with increased periods of exposure to NGF, indicating that NGF transiently activated a receptor-related protein kinase. If cytoskeletons were prepared after a 5 min. exposure to NGF and the phosphorylation reaction carried out for different periods of time, a time-dependent increase in labeling of the 250 kdalton protein was observed. These data indicate that the receptor-stimulated kinase, once activated, is a stable species.

The effect of NGF was half-maximal below 38pM, consistent with the presence of the high affinity form of the NGF receptor. Addition of NGF directly to cytoskeletal preparations had no effect.

These data suggest that binding of NGF to its cell surface receptor results in rapid, but transient, activation of a cytoskeletally-associated protein kinase coincident with the attachment of the hormone-receptor complex to the cytoskeleton. The receptor-related protein kinase preferentially phosphorylates a 250 kdalton protein whose identity is under investigation.

320.7 DEVELOPING RAT OPTIC NERVES SYNTHESIZE A SPECIFIC SUBSET OF GROWTH-ASSOCIATED PROTEINS. Susan Bock*, Jeanette J. Norden, G. Jackson Snipes*, and John A. Freeman (SPON: A. Burt). Dept. of Anatomy, Vanderbilt University, Nashville, TN 37232.

The failure of the optic nerves of mammals to regenerate following injury stands in contrast to the ability of the optic nerves of lower vertebrates to regenerate and restore vision. In toads and goldfish the synthesis of a specific subset of rapidly transported growth-associated proteins (GAPs) is selectivley enhanced during regeneration, suggesting that the ability of CNS neurons to regenerate may depend upon the ability to induce a critical class of proteins (Skene and Willard, 1980). If in general, the growth of axons is regulated by such proteins, one would expect them to be expressed during development as well. We have examined developmental changes in the expression of rapidly transported proteins in the optic nerves of neonatal rats during the 10 day post-partum period when optic nerve fibers are in a state of active growth and maturation (Lund and Bunt, 1976). Rapidly transported proteins were labeled by the intraocular injection of ^{35}S-methionine and resolved using 2-dimensional gel electrophoresis with flourography. A number of computer algorithms employing video graphics analyses were devised to facilitate quantitation and comparison. We found that the total protein incorporation and transport was dramatically greater in new-born rats, decreasing towards the lower adult level by day 10 post-partum. In contrast to a spectrum of several hundred polypeptides whose relative expression appears to remain constant over time, there is a limited class of polypeptides whose expression undergoes characteristic developmental changes. The majority of proteins that are selectively expressed during the first 10 days post-partum have an acidic pI. Dramatic changes occur in proteins having a molecular weight (in kilodaltons) of 18k, 24k, 55k, and 66k; their expression initially high, falls to a very low level by day 10, while the synthesis of a 14k and of a 30k polypeptide increase during this same period. The 18k and 24k polypeptides appear to be homologous to similar GAPs expressed during the regeneration of toad and goldfish optic nerves. Efforts are presently being directed at obtaining monoclonal antibodies against these various growth associated proteins in order to establish possible inter-species homology, and to determine their subcellular localization. It appears that axonal development, as well as regeneration, is associated with a specific subset of proteins.

Supported by NIH grants EY01117 and NS 18103 to J.A.F.

320.8 DEVELOPMENT OF RAT RETINAL NEURONS IN MONOLAYER CULTURE: EFFECT OF ELEVATING THE CONCENTRATION OF K^+ IN THE GROWTH MEDIUM. B-A. Battelle, M.E. Truckenmiller* and J.W. Pepper*. National Eye Institute, NIH, Bethesda, MD. 20205.

The development of synaptic function of mammalian retinal neurons is being studied using cells dissociated from retinas of embryonic rats and grown in monolayer culture. The time courses of development of GABA and ACh synthesis and storage have been measured in these cultures and they differ considerably from one another. Significant ACh synthesis was detected after 1 day in culture and the rate of ACh synthesis increased 7 to 10-fold during the first week. During the second week in culture, the rate of ACh synthesis fell sharply to a level similar to that measured after 1 day in culture. GABA synthetic activity, on the other hand, was barely detectable between 1 and 3 days in culture. It then increased 30-fold during the next 10 to 12 days and remained high for at least a week. These results suggest that the requirements are different either for the survival of the two biochemical classes of retinal neurons or for the maintenance of their neurotransmitter synthetic activity.

Growing rat retinal cells in medium containing a high concentration (50 mM) of K^+ markedly changed the appearance of the cultures. The processes of some cells became thicker and much more prominent, and there was an apparent increase in the number of cells with large somata (15 to 20 um in diameter). Retinal cells grown for 2 to 3 weeks in elevated K^+ were examined using the monoclonal antibody A2B5 which was developed as a specific marker for chick retinal neurons by Eisenbarth et al. (PNAS 76: 4913, 1979). The antibody was applied to living cultures and the location of the antibody was visualized using indirect immunofluorescence. A2B5 bound to cell bodies and processes of some but not all of the large neuron-like cells. Some flat cells also were labeled with A2B5.

The rate of GABA synthesis in cultures grown in the presence of elevated K^+ was twice that measured in sister cultures grown in normal medium. An increase in the rate of GABA synthesis could be measured within 24 hours after switching cells that had been grown for two days in normal medium into medium containing 50 mM K^+. In cultures grown for two weeks in elevated K^+, the number of neuron-like cells which took up radiolabeled GABA was 3 to 4 times higher than in control cultures. The total number of neuron-like cells in high K^+ and control cultures was similar. Experiments are in progress to determine 1. if elevating the concentration of K^+ in the growth medium causes a general enhancement of neurotransmitter synthetic activity or if its effects are specific for GABAergic cells and 2. if the increase in GABA synthetic activity results from selective survival of GABAergic cells in culture or an increase in GABA synthetic activity per cell.

320.9 MORPHOLOGICAL AND BIOCHEMICAL CORRELATES OF THE DEVELOPING SQUID RETINA: STUDIES USING PHOTORECEPTOR SPECIFIC ANTIBODIES. Fulton Wong*, Cheng-Chien Song*, Roger Hanlon* and Raymond Hixon* (SPON: N. Kremer). Marine Biomed. Inst. and Dept. of Physiol. & Biophys., Univ. TX Med. Br., Galveston, TX 77550.

We have previously isolated a set of monoclonal antibodies that are specific for squid photoreceptors and have identified the associated antigens. As a prelude to studies designed to establish the functions of these photoreceptor specific antigens, we have examined the developmental profiles of two of these antigens using the technique of indirect immunofluorescence. One of the antigens is the visual pigment rhodopsin, the function of which is to capture light. The other is a newly identified protein called SP45. In the adult retina, SP45 is shown to have several interesting properties that suggest a possible role in transduction.

Retinas of embryos in a late developmental stage are known to be functional. However, the morphology of the retina at this stage is quite different from that of the adult. The most noticeable difference is that the outer and inner segments of the photoreceptors are of equal length. Furthermore, these segments of the cells are separated by a layer of glial cells. Such an organization gives the retina an appearance of having three distinct layers each of approximately the same thickness. At this late stage, both antigens are found to appear most abundantly at the inner segments. This is consistent with high rates of synthesis of these antigens and also indicates that the antibodies could recognize the newly synthesized molecules.

Studies of earlier embryonic stages indicate that both antigens appear very early in the development of the photoreceptors. For example, rhodopsin appears in the outer segments as soon as the outer segments are visible and identifiable. As in the adult, the protein SP45 is found to exist throughout the entire photoreceptor, including the axon.

Information obtained from these studies will provide the groundwork for future studies on the expression of the genes that code for these proteins. Since the antibodies can recognize the photoreceptors at very early stages of development, they will also be useful as cell markers to study dissociated cells and monitor the development of photoreceptors in vitro.

(Research supported by NSF BNS-07067, NIH EY-03308 and NIH NS-11255.)

320.10 DEVELOPMENT OF SEROTONERGIC AND ADRENERGIC RECEPTORS IN THE RAT SPINAL CORD: EFFECTS OF NEONATAL CHEMICAL SYMPATHECTOMY. C. Lau, A. Pylypiw* and L. L. Ross. Dept. Anatomy, The Med. Coll. of Pennsylvania, Phila., PA 19129.

The sympathetic preganglionic neurons in the spinal cord receive dense serotonergic (5-HT) and catecholaminergic (CA) afferent inputs from the descending supraspinal pathways. In the neonatal rat spinal cord, the levels of these biogenic amines and their receptors are low at birth, but undergo rapid ontogenetic increases in the ensuing 2-3 postnatal weeks until the adult levels are reached. In many systems it has been shown that denervation of presynaptic neurons leads to an up-regulation of the number of postsynaptic receptors. To determine whether the 5-HT and CA receptors in the developing spinal cord are also subject to such transsynaptic regulation, we examined the ontogeny of serotonergic receptors and α- and β-adrenergic receptors in thoracolumbar spinal cord of rats given neurotoxins which destroy serotonergic (5,7-dihydroxytryptamine (5,7-DHT) or noradrenergic (6-hydroxydopamine (6-OHDA)) nerve terminals. Intracisternal administration of 5,7-DHT or 6-OHDA at 1 and 6 days of age prevented respectively the development of 5-HT and CA levels in the spinal cord. In controls, specific 5-HT receptor binding measured with (^{3}H)-5-HT was approximately 0.1 pmol/spinal cord at 1 day of age, rose to 0.2 pmol at 7 days and reached adult levels (0.5 pmol) by 2 weeks postnatally. Rats lesioned with 5,7-DHT displayed a marked elevation of 5-HT receptors with a binding of 50% greater than controls at 1 week and a continuing increase to twice normal by 4 weeks. A similar pattern of up-regulation was also detected with the α-adrenergic receptor (measured by specific binding of (^{3}H)-prazosin), as rats lesioned with 6-OHDA showed persistent increases in receptor concentration. However, in these same animals ontogeny of the β-adrenergic receptor in the spinal cord (measured by specific binding of (^{3}H)-dihydroalprenolol) remained virtually unaffected by the chemical lesion. These results suggest that: (a) development of the postjunctional serotonergic and α-adrenergic receptors in the rat spinal cord can occur in the absence of the prejunctional nerve terminals and are subject to transsynaptic modulation; (b) β-adrenergic receptors in the spinal cord also can develop after prejunctional lesions but are independently regulated.

Supported by the Office of Mental Health of the Commonwealth of Pennsylvania.

320.11 REGULATION OF TYROSINE HYDROXYLASE (TH) DEVELOPMENT IN THE LOCUS COERULEUS (L.C.) IN VIVO AND IN CULTURE. K.A. Markey, C.F. Dreyfus and I.B. Black. Division of Develop. Neurol., Cornell University Medical College, New York, New York 10021.

To begin defining translational and post-translational mechanisms involved in development of brain transmitter phenotypic traits, the mouse L.C. was examined. Physico-chemical properties of TH, the rate-limiting enzyme in catecholamine synthesis, were characterized in vivo and in explant culture.

TH is initially detectable catalytically and immunohistochemically on E13 (gestational day) in L.C. and reaches adult values by postnatal day 18 in vivo (Devel. Biol., in press, 1983). Explantation of the L.C. primordium on E12 results in the de novo appearance of TH, but the increase in activity does not reach normal adult levels. In contrast, E18 explants approximate normal adult levels of TH after 3 weeks in culture.

Immunotitration studies indicated that the developmental increases in locus TH activity in vivo and in vitro were attributable to increased numbers of enzyme molecules. Moreover, comparative thermostability experiments indicated that gross TH structure did not change during development: catalytic activity was reduced by 50% in a 3 minute incubation at 57°C for adult, E18 and cultured neurons.

Under physiological conditions, TH must be activated to function normally, and activated and non-activated species differ in affinity for cofactor and pH optima. Lineweaver-Burke analyses in our studies indicated that affinity for the BH_4 cofactor did not change significantly during development (Adult K_m=130 μM; E18 K_m=170μM). However, comparison of pH optima revealed a shift toward more activated TH during development in vivo: the ratio of activities at pH 7 to pH 6 shifted from 0.39± 0.03 to 0.58 ± 0.02 from E18 to postnatal day 60. A similar shift occurred in E18 explants cultured for 2 weeks. In contrast, no shift occurred in cultured E12 L.C., which exhibited a ratio of 0.31 after 2 weeks. Consequently, de novo TH expression and developmental enzyme activation are regulated by distinct processes.

Our observations suggest that the developmental increase of TH in L.C. is due to accumulation of enzyme molecules without any gross change in protein structure. However, TH does not undergo detectable activation during development which may increase catalytic function. Thus, a combination of translational and post-translational mechanisms govern development of this transmitter character in this brain nucleus.

(Supported by NIH grants BRSG RR 05396, NS 10259 and HD 12108, NSF BNS 8024081).

320.12 STRIATAL UNIT ACTIVITY CHANGES IN DEVELOPING RATS FOLLOWING SINGLE OR REPEATED HALOPERIDOL ADMINISTRATION. T. C. Napier, S.Coyle*, and G. R. Breese. Biological Sciences Research Center, UNC School of Medicine, Chapel Hill, NC 27514.

The caudate nucleus (CN), or striatum, contains cells which are postsynaptic to dopamine (DA) neurons. Neuroleptics' effects on DA systems are often reflected as changes in unit activity in the CN. Since behavioral and biochemical measures indicate that, in rats, DA systems continue to develop postnatally (see previous abstract), in the present study, we examine the effects of haloperidol on neuronal activity recorded from the CN of rat pups.

Offspring of Sprague-Dawley rats bred in our colony served as subjects. Treatment began when litters were 1, 10 or 21 days of age, with testing occurring one week later. For 7 days, half the pups in each litter were treated with haloperidol (1 mg/kg, i.p.), while the remaining pups were injected with an equal volume of saline vehicle. On the eighth day, animals from each treatment group were tested with either saline or haloperidol (1 mg/kg).

For recording single unit activity, the animals were anesthetized with chloral hydrate. To facilitate stereotaxic procedures, 7 & 17 day old pups were placed in a form-fitted mold (Lithgow & Barr; Dev Brain Res. 2:315, 1982), while 28 day olds were tested in a modified mouse stereotaxic instrument. Spontaneously-firing, single unit activity was located in CN and the signal was amplified and recorded using standard electrophysiological procedures.

In all age groups tested, the saline treated animals had the fewest active cells. Acute administration of haloperidol increased the number of spontaneously active cells in each age group. Spontaneously firing cells were more frequently observed in older pups, as were cells which demonstrated bursting activity. By 28 days of age, animals tested following repeated haloperidol administration showed a decrease in the number of observed cells compared to subjects tested after an acute dose of haloperidol. More cells were encountered in animals tested with saline following repeated haloperidol administration than in saline controls. Also, the number of cells observed in the former group was similar to that seen in subjects tested after acute haloperidol. These data were unexpected since behavioral and biochemical measures for this treatment and age group were at control levels (see previous abstract). Further experimentation is necessary to more clearly define the effects of haloperidol in developing animals.

(Supported by USPHS Grants NS-07166, HD-03110 and Scottish Rite Schizophrenia Research Program NMJ, USA.)

320.13 SENSORY INNERVATION REGULATES THE LEVEL OF CHOLINERGIC RECEPTORS ON INSECT CENTRAL NEURONS DURING POSTEMBRYONIC DEVELOPMENT. M.R. Meyer, G.R. Reddy*, and J.S. Edwards. Dept. Zoology, NJ-15, Univ. of Washington, Seattle, WA 98195.

During postembryonic development of identifiable target giant interneurons (GI) in the terminal abdominal ganglion (TG) of the cricket, chronic deafferentation leads to marked reduction of dendritic growth, decreased intracellular protein metabolism, and altered physiological response of GI. In contrast, mature GI do not display such changes in response to removal of presynaptic input. These findings suggest that sensory neurons exert inductive effects upon growth and development of postsynaptic central target via regulation of cellular metabolism (Meyer and Edwards, J. Neurosci, 2:1561, 1982). To examine this more closely, the effects of deafferentation on TG cholinergic sites, which are thought to be localized in the cercal sensory-GI pathway in orthopteran insects, were investigated.

Incubation of TG homogenates with the labeled muscarinic antagonist [^{3}H]-1-QNB discloses a unique class of binding sites which display mixed muscarinic-nicotinic properties. A binding site density (B_{max}) of 1.9±0.4 pmol/mg protein with an apparent dissociation constant (K_d) of 9.9±3.0x10^{-9}M was calculated from Scatchard analysis of binding isotherms. One week following bilateral deafferentation of adult TG by removal of cercal sensory organs, no noticeable changes were found for either B_{max} or K_d. After two weeks, only slight decreases in B_{max} were noted, even though most presynaptic terminals on GI are lost within 24 hours of deafferentation. In marked contrast to short-term treatment, when TG were chronically deafferented throughout postembryonic development, a 40% decrease in B_{max} (with no alteration of K_d) occurred.

Autoradiographic studies are now in progress in order to localize cholinergic binding sites within the cercal sensory-GI pathway and to compare receptor labeling patterns between control and deafferented regions of TG neuropile in unilaterally cercectomized animals.

That these data for [^{3}H]-QNB binding to TG sites closely parallel changes in growth and protein metabolism of target GI following long-term removal of afferent neurons suggests that presynaptic influences may be important for metabolic regulation of central postsynaptic macromolecules intimately involved in synaptic function. Supported by NIH NB-07778 and UWGSRF.

320.14 RATS GIVEN DOPAMINE-DEPLETING BRAIN LESIONS AS NEONATES ARE SUBSENSITIVE TO DOPAMINERGIC ANTAGONISTS AS ADULTS. John P. Bruno, Edward M. Stricker and Michael J. Zigmond. Depts. of Psychology, and Biol. Sciences, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Rats sustaining large 6-hydroxydopamine (6-HDA)-induced dopamine (DA)-depleting brain lesions as adults initially exhibit akinesia, catalepsy, and aphagia. After a gradual recovery of function, the dependence of behavior on residual DA activity is illustrated by their continued sensitivity to DA antagonists. Indeed, doses of these drugs that have no obvious effect in intact rats reinstate severe behavioral impairments in the brain-damaged animals. We have previously reported that rats given brain lesions as neonates fail to show initial dysfunctions (Bruno et al., Soc. Neurosci. Abstr. 7: 168, 1981). The present experiments examined whether these rats remained sensitive to the behavioral effects of DA antagonists.

Three days after birth, rats received 150 ug 6-HDA or its vehicle into the lateral cerebral ventricles, 30 min after treatment with desmethylimipramine (25 mg/kg, ip). Behavioral testing was carried out 5-8 months later. First animals were tested for akinesia (latency to move all four paws) and catalepsy (latency to remove front paws from a wooden block) before and after administration of the neuroleptic, haloperidol (0.6 mg/kg, ip).

	AKINESIA (sec)		CATALEPSY (sec)	
	Pre	Post	Pre	Post
Vehicle	3.2 ± .6	62.3 ± 14.0	4.2 ± 1.4	90.3 ± 19.8
6-HDA	4.8 ± 1.8	5.1 ± 2.6	17.4 ± 4.1	15.5 ± 4.1

These data were obtained 2 hr after haloperidol treatment, but similar effects were observed throughout the 4-hr period. All brain-damaged animals had DA depletions of at least 98%.

In addition, we examined the effects of a DA antagonist on food intake in rats adapted to a 4 hr/day feeding regimen. Brain-damaged animals were not affected by treatment with fluphenazine (0.2 mg/kg, ip) whereas the food intakes of control rats were significantly depressed (the groups ate 85% and 36% of basal intakes, respectively; $p<.01$).

Thus, rats given large DA-depleting brain lesions as infants are much less sensitive to effects of DA antagonists than are rats given comparable brain lesions as adults. These observations suggest that nondopaminergic systems may influence ingestive and motor behavior after neonatal brain damage. In this regard, we are currently studying whether the sprouting of striatal serotonin-containing neurons, which occurs after 6-HDA treatment in infant rats but not in adults (Stachowiak et al., Soc. Neurosci. Abstr. 8: 304, 1982), is involved in the mediation of these behaviors.

(Supported by research grant NS 16359 and MH 30915.)

320.15 ONTOGENY OF BEHAVIORAL AND BIOCHEMICAL TOLERANCE TO REPEATED ADMINISTRATION OF HALOPERIDOL. S. Coyle*, T. C. Napier and G.R. Breese. (SPON: S. K. Burgess). Biological Sciences Research Center, UNC School of Medicine, Chapel Hill, NC 27514.

In the majority of reports on the effects of repeated administration of neuroleptics to neonatal rats, investigators have waited to test the pups until they are at least of weaning age. While this approach determines some of the long lasting effects of neonatal neuroleptic treatment, it does little to define how infant rats respond to neuroleptics during treatment. Since dopamine (DA) systems do not become mature until after the first postnatal month, we hypothesized that infant rats would not respond to repeated administration of haloperidol in the same manner as do adults. To test this hypothesis, in these studies, we examined the ontogeny of behavioral (catalepsy) and biochemical tolerance following repeated administration of haloperidol.

Offspring of Sprague-Dawley rats bred in our colony served as subjects. Treatment began when litters were 1, 7, 14, 21 or 28 days of age, with testing occurring one week later. For 7 days, half the pups in each litter were treated with haloperidol (1 mg/kg, i.p.), while the remaining pups were injected with an equal volume of saline. On the eighth day, animals from each treatment group were tested with either saline or haloperidol. Pups were tested for the presence of catalepsy 15, 30, 45, and 60 min. after injection. Seventy min. after the injection, subjects were decapitated and the corpus striatum removed for HPLC determination of biogenic amines and their metabolites.

In interpreting the results from the behavioral observations, some considerations which must be included are: 1) Do animals of a particular age show catalepsy in response to an acute injection of haloperidol? and 2) If haloperidol induces catalepsy after acute administration, do animals treated repeatedly with haloperidol become tolerant to its cataleptogenic effects? The results, summarized below, not only indicate a developmental change in the response to acute and repeated administration of haloperidol, but also a dissociation between behavioral and biochemical measures.

AGE(days)		BEHAVIOR	BIOCHEMISTRY		
Treat	Test	(Catalepsy)	DOPAC/DA	HVA/DA	5HIAA/5HT
1-7	8	+/no Tol	NC	+/no Tol	NC
7-13	14	NC	NC	+/no Tol	NC
14-20	21	+/Tol	+/no Tol	+/no Tol	NC
21-27	28	+/Tol	+/no Tol	+/no Tol	NC
28-34	34	+/Tol	+/Tol	+/Tol	NC

"+" increase over controls; "Tol" repeated-haloperidol different from acute-haloperidol; "NC" haloperidol not different from saline.

(Supported by Scottish Rite Schizophrenia Research Program, NHJ, USA and USPHS Grants NS-07166 and HD-03110.)

320.16 IN VITRO ^{3}H-SEROTONIN AND ^{3}H-DOPAMINE UPTAKE INTO DISSOCIATED EMBRYONIC NEURONS AND ADULT FIBROBLASTS. E. Lieth*, A. C. Towle*, T. H. Joh[1] and J. M. Lauder. Anatomy Dept., UNC Sch. Med., Chapel Hill, NC, and [1]Cornell Univ. Med. Coll., New York, NY.

We have sought to examine possible interactions between identified developing neurons in culture. To assess the interactions we have measured monoamine uptake and performed semi-quantitative immunocytochemistry in parallel embryonic rat brain cultures. Cultures of dissociated embryonic brain (E14) were maintained on poly-L-lysine for 4-6 days. Cultures were then either fixed with 4% paraformaldehyde for serotonin or tyrosine hydroxylase immunocytochemistry, or used to determine ^{3}H-serotonin (5HT) or ^{3}H-dopamine (DA) uptake. Distinct high affinity, energy dependent, saturable 5HT and DA uptake systems were present in the cultures and had properties similar to those described in adult tissues.

Brain cells cultured from mes- or metencephalon of E14 rats (containing developing substantia nigra and raphe nuclei, respectively) were incubated with 3.8×10^{-8} M ^{3}H-5HT or 2.6×10^{-8} M ^{3}H-DA for 30 minutes at 37°C. Preliminary results indicate a significant enhancement of DA uptake when the two tissues were cultured together as compared to each region alone, while 5HT uptake was only slightly enhanced using the same conditions. The DA effect is interesting, since asonal projections from the raphe nuclei reach the substantia nigra by E13-14, raising the possibility that the increased DA uptake is a manifestation of this interaction. A similar enhancement of DA uptake was observed in dissociated E14 mesencephalon maintained on a monolayer of purified astrocytes, in contrast to normal rat kidney fibroblasts (NRKs). The latter effect may be due to better survival, growth and differentiation of neurons on their native glial substrate.

Interestingly, when we attempted to compare the growth of 5HT neurons on NRKs or astrocytes, we found that NRKs alone had a significant level of ^{3}H-5HT uptake. NRK fibroblasts exhibited a high affinity uptake system that was blocked by 100 fold excess fluoxetine. ^{3}H-DA was not taken up under similar conditions. The importance of 5HT uptake into fibroblasts remains unknown.

Combined autoradiographic-immunocytochemical investigations are presently in progress to examine the specificity of the uptake of ^{3}H-monoamines into identified 5HT and DA neurons in these cultures. Experiments to determine the possibility for 5HT influences on the enhancement of DA uptake in the co-cultures are currently being evaluated. NS-15706, NS-07166, NS-00507.

320.17 DEVELOPMENT OF CYTOPLASMIC ESTROGEN AND PROGESTIN RECEPTORS IN BRAIN OF FEMALE GUINEA PIG. H.I. RYER* AND H.H. FEDER* (SPON: R. ERICKSON). Institute of Animal Behavior, Rutgers University, Newark N.J. 07102.

The adult female guinea pig is dependent on the action of estrogen and progesterone (P) for the display of receptive behavior (lordosis). However, during early postnatal development the lordosis response appears to be insensitive to the priming action of these hormones. In the following experiments cytoplasmic estrogen and progestin receptors were examined in the brains of neonatal female guinea pigs to determine if attainment of sensitivity to estrogen action on lordosis response is correlated with developmental changes in these receptor systems.

Ovariectomized neontal (7 day old) guinea pigs failed to display lordosis after priming with 10μg estradiol benzoate (EB) and .5mg P. Even at 29-33 days of age a significantly smaller percentage of females displayed lordosis as compared to adult females

Neither the concentration, nor the affinity of cytoplasmic estrogen receptors (CER) in neonatal hypothalamus (HYPO), preoptic area (POA), amygdala (AMYG) or cortex (CORT) were found to be significantly different from that found in adult brain areas.

The concentration of cytoplasmic progestin receptors (CPR) was compared in neonatal and adult HYPO, POA and CORT 40h after an injection of oil or EB. For all three brain areas, the concentration of CPR in unprimed neonatal guinea pigs was significantly lower than that found in adults. Neonatal females were injected with both low (1.6μg) and high (10 and 50μg) doses of EB and compared to adult females given a low (1.6μg) but behaviorally effective dose of EB. HYPO and POA but not CORT showed estrogen dependent induction of CPR. In neonatal HYPO both low and high doses of EB failed to induce a concentration of CPR significantly greater than that seen in adult female HYPO after a low dose of EB. Neonatal and adult POA did not show significantly different concentrations of CPR at a low dose of EB. High doses of EB resulted in CPR concentrations in neonatal POA significantly greater than that seen in adult POA.

These experiments reveal that neonatal behavioral insensitivity to estrogen is accompanied by deficits in the action of estrogen in female HYPO but not POA as indicated by reduced levels of CPR induction.

320.18 **REFLEX DEVELOPMENT IN MICE PRENATALLY EXPOSED TO PHENCYCLIDINE[1].** **T. A. Fico and C. VanderWende. Rutgers University, Box 789, Piscataway, New Jersey 08854.**

Phencyclidine (PCP) has been shown to delay the development of some reflexes in mice when administered during gestation and lactation (J.M. Nicholas, et al., Fed Proc., 40 : 298, 1981). To investigate whether the above reported delay in reflexontogeny was due to prenatal exposure to PCP or neonatal exposure to the PCP present in mothers milk (J. M. Nicholas, et al., AM. J. Obstet. Gynecol., 143: 143, 1982), we examined the development of the righting reflex in the offspring of mothers given PCP only during gestation. Nulliparous female CF-1 mice were injected (SC) with either saline, 5, 10, 20 or 40 mg/kg of PCP from day 6 of gestation through and including day 15. Birth weight and righting time were determined within 24 hours of birth. Prenatal exposure to PCP had no effect on the righting time of the male offspring. The female offspring prenatally exposed to 10, 20 or 40 mg/kg PCP had an increased latency to righting themselves, however, this was not significant. These results indicate that prenatal exposure to PCP does not adversely effect development of the righting reflex and the retardation reported by Nicholas, et al., was probably due to neonatal exposure to the drug.

[1]Supported by the Charles and Johanna Busch Foundation.

320.19 THE DEVELOPMENT OF MUSCARINIC BINDING SITES IN NORMAL DEVELOPING AND TRANSPLANTED MOUSE NEOCORTEX. C.F. Hohmann, C.B. Pert, F.F. Ebner. Div. of Biol. and Med., Brown Univ., Prov., R.I.02912 and Lab. of Clin. Sci., NIH/NIMH, Bethesda, Md.20205.

We reported previously that cholinergic markers develop in transplanted neocortex as in normal cortex, with the difference that AChE-staining in postnatal donor cases develops a much denser pattern than in prenatal donor transplants where AChE is far below host levels. These results raise the question of whether muscarinic cholinergic receptors are present in different densities at the time of transplantation and whether they develop a differential density in pre- and postnatal donor tissue after differentiating for 30 days in an adult host brain.

Tritiated Propylbezilylcholine-mustard, a permanent muscarinic binding agent was used to label muscarinic receptor sites. Displacement of label by atropine was used as a control for the specificity of the binding. Autoradiography was performed on coronal brain sections of normal animals ranging in age from E18 over various postnatal ages to adult, and on neocortical transplants of various donor ages.

In normal animals, some mustard binding can be observed in cortex as early as E18. Binding slowly increases during the first postnatal week and then rapidly gains in intensity during the second week. The pattern of mustard binding changes slightly during the course of development. Expression of the mature pattern of mustard binding autoradiography is reached by 3 weeks postnatal. Thus in normal animals the development of muscarinic receptor sites slightly preceeds the expression of AChE.

High grain densities were seen in all neocortical transplants after one month postoperative survival. Mustard binding in prenatal donor tissue was equal to or slightly higher than in surrounding host cortex. Binding in postnatal donors was equal or slightly lower than in the host tissue. The transplants thus develop an approximately normal number of receptor sites for one month old tissue.

These results differ from our findings in the AChE study where the appearance of AChE positive fibers in the transplant tissue varies with the age of the donor at the time of transplantation. Prenatal donor tissue never reached a density of AChE stain comparable to adult cortex. We conclude that the expression of muscarinic receptor sites in transplant tissue and the ingrowth of AChE positive fibers are not positively correlated and thus are likely to be regulated by separate mechanisms. (Supported by grant #NS-13031)

DISEASES OF SYNAPSES AND AXONS

321.1 MOTOR NERVE ENDING REPETITIVE DISCHARGES AS A MEASURE OF NEURAL EXCITABILITY IN INTACT AND ADRENALECTOMIZED RATS. J. Sprouse* †, T. Baker and W.F. Riker. Depts. of Pharmacology & Anesthesiology, Cornell Univ. Med. Col., New York, N.Y. 10021

Recent studies from this laboratory demonstrated that motor nerve ending (MNE) excitability is depressed in adrenalectomized rats. In those experiments, neostigmine-induced MNE repetitive discharges, quantitated as fasciculatory muscle action potentials, served as an indirect indicator of MNE excitability in vivo. Treatment of adrenalectomized rats with the mineralocorticoids, aldosterone or desoxycorticosterone acetate, largely restored this measure of excitability to normal. The concurrent lowering of an elevated plasma K^+ by these mineralocorticoids suggested that K^+ levels alter MNE excitability.

The experiments reported here represent a more direct readout of MNE repetition. Specifically, the antidromic component of the MNE fasciculatory discharge was monitored with electrodes placed at the ventral roots. Compared to intact controls, the total number of antidromic discharges following neostigmine (0.10 mg/kg i.p.) was reduced to approximately 20% in adrenalectomized rats ($p < 0.05$); a similar decline in their peak rate of occurrence was also observed. Consequently, the depression in MNE excitability shown in adrenalectomized rats in the previous study was confirmed. If these changes in MNE excitability are related to plasma K^+, it should be possible to restore excitability to normal by simply restoring plasma K^+ to normal. To test this hypothesis, adrenalectomized rats were placed on a K-deficient diet ad lib.for 5-7 days. At the end of this period, plasma K^+ was lowered (8.6 to 5.5 mEq/l) and the fasciculatory response to neostigmine increased by approximately 4-fold ($p < 0.05$).

To ascertain whether these changes in MNE excitability were indicative of similar changes occurring along the length of the peripheral motor nerve, strength-duration relationships were determined on rat sciatic nerve in vivo, yielding values of chronaxie and Blair's rate constant. According to these measures, axonal excitability was reduced in adrenalectomized rats; lowering plasma K^+ in adrenalectomized rats by dietary restriction restored excitability.

From these studies it is concluded that (1) the peripheral motor nerve from its exit out of the spinal cord to its unmyelinated terminals is less excitable as a result of adrenalectomy; (2) an increase in plasma K^+ following adrenalectomy accounts for the loss of excitability in motor nerves; (3) MNE repetitive activity measured as either fasciculatory muscle action potentials or antidromic discharges reflects these excitability changes.

† Recipient, Norman and Rosita Winston Foundation Research Fellowship.

321.2 CONDUCTION IMPAIRMENT INDUCED BY LOCALIZED COOLING OF IN VITRO RAT TAIL NERVE DETECTED BY HIGH FREQUENCY TRAINS OF STIMULI. T.C. Chimento*, D.L.Jewett, and G. Heard* (SPON: J.Williston). Dept. of Orthopaedic Surgery, Special Studies Unit, Univ. Cal. San Francisco, San Francisco, CA. 94143.

A rat tail nerve suspended on Ag/AgCl wire electrodes in a mineral oil bath was subjected to localized temperature changes along a 30 mm region between the stimulating (S1)and recording electrode. Stimulus trains of increasing frequency (STIF), uniform frequency (STUF) and decreasing frequency (STDF) were presented to the distal end of the nerve and recorded monophasically. Height, area and several latency endpoints were used to examine the combined effects of supramaximal stimulation presented in the relative refractory period with cooling of a central region of the nerve. The latency of a point on the rising phase at 1/2 the peak amplitude of a response was chosen as the most sensitive and reliable endpoint.

As temperature was decreased the latency of responses to high frequency stimuli increased more rapidly than the response to the first stimuli of a train. A second pair of stimulating electrodes (S2) were placed on the far side of the cooling bath to monitor the nerves response during the experiment to be sure the effects seen at S1 were caused by the temperature changes and not some general change in the nerve or a change at the recording electrodes.

The STDF, which is a mirror image of the last 8 responses in a STIF, was used to demonstrate cummulative effects on latency of responses at the end of a STIF. Although the interval preceeding the ninth response in a STIF was equal to the interval preceeding the third response in a STDF the latency was consistantly less in the STDF. The effect of repeatedly stimulating in the relative refractory period is to produce increasing latency from successive responses in a train. The STUF pattern demonstrates that this latency increase usually assymptotes after several stimuli of equal interval are presented. The latency may cease to increase after 5 to 8 stimuli depending on the frequency of the train and the temperature of the cooled region.

The duration of the last response in a STIF was measured from the 1/2 amplitude of the rising to the falling phase of the wave. No change was noted as the temperature was decreased. This endpoint placed our cursors above the bump on the falling phase that represents a somewhat slower population of fibers.

This data illustrates that using high frequency trains of stimuli which force successive responses to occur in the relative refractory period of the preceeding response is a more sensitive method of detecting localized conduction impairment than using a single stimuli.

321.3 DEMYELINATED NERVE FIBERS: BURST ACTIVITY FOLLOWING BLOCKAGE OF G_K. S. G. Waxman, J. D. Kocsis, D. L. Eng* and R. J. Preston. Dept. of Neurology, Stanford Univ. Medical School, and Veterans Administration Medical Center, Palo Alto, CA 94304.

In normal mature mammalian myelinated fibers, voltage-sensitive potassium conductance (g_K) does not contribute to repolarization of the action potential, and application of the g_K-blocking agent 4-aminopyridine (4-AP) does not alter action potential waveform. In contrast, when g_K is blocked with 4-AP in immature myelinated fibers, burst activity is elicited by a single stimulus (Kocsis et al, J. Neurophysiol., in press); in regenerated rat sciatic nerve following myelination, blockage of g_K with 4-AP similarly leads to bursting in response to a single stimulus. In this study, chronic demyelinating lesions were produced in the sciatic nerves of adult Wistar rats by placement of loose ligatures. Three to 97 days after production of the lesion, nerves were excised and studied in a modified sucrose gap chamber in which whole nerve responses and membrane potential changes could be examined, and in which intra-axonal impalements could be obtained with glass microelectrodes. Following treatment of nerves with either of the aminopyridine analogs, 4-AP or 3,4-diaminopyridine (3,4-DAP), the response to single stimuli was altered. The compound action potential changed from a single large amplitude negativity to one which included late negative components that gave the field a rippled appearance. This late activity was shown, by both occlusion experiments and from intra-axonal recording, to correspond to burst activity in single fibers in response to single stimuli. In contrast, when bathed in normal Ringer solution in the absence of 4-AP or 3,4-DAP, axons responded with a single action potential but did not burst in response to a single stimulus. Histological examination indicated that the pathology of the studied nerves was characterized by demyelination and remyelination. These results support the proposals that potassium channels may be localized in non-nodal axolemma; and that these channels play a role in determining firing properties of demyelinated and remyelinated fibers. Supported in part by the National Multiple Sclerosis Society and the Veterans Administration.

321.4 EFFECTS OF TRIMETHYLTIN ON THE HAMSTER STELLATE GANGLION. D. Christ, L. Chang and D.E. McMillan*. Dept. of Pharmacology and Interdisciplinary Toxicology and Dept. of Pathology, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205.

Trimethyltin (TMT), at doses of 3 or 4 mg/kg, i.p., produced a marked tremor in hamsters within 24 hrs after administration. No tremor was observed 24 hrs after 2 mg/kg TMT. Stellate ganglia were isolated from control hamsters and hamsters pre-treated with TMT (4 mg/kg) for 24 hrs. The ganglia were mounted in a chamber for extracellular recording of action potentials. There were no differences in the compound action potentials (0.2 Hz or trains at 30 Hz for 2 sec) from ganglia of control and TMT hamsters. Afterdischarges were induced by preganglionic stimulation at 30 Hz for 2 sec (1 train/min) in the presence of 10^{-3} M hexamethonium. The afterdischarges in ganglia from TMT hamsters were less than 10% of the afterdischarges from ganglia of control hamsters. The discharges induced by McN-A-343, a muscarinic cholinergic agonist, were also much smaller in the ganglia from TMT hamsters. Post-train enhancement of the afterdischarges or the McN-A-343 discharges was not changed by TMT. These results suggest that TMT blocks muscarinic transmission in the stellate ganglion. Muscarinic mechanisms are also involved in two other phenomena in the stellate ganglion - post-tetanic potentiation (p.t.p.) of the compound action potential in the presence of hexamethonium and potentiation of the compound action potential in hexamethonium by McN-A-343. The p.t.p. of the compound action potential was smaller in ganglia from TMT hamsters, but the effect of TMT on p.t.p. was much less than the effect on the afterdischarges. There was no difference between the magnitudes of the McN-A-343 potentiation in ganglia from control and TMT hamsters. These results indicate that TMT reduces the muscarinic processes involved in the production of the afterdischarges but has only a small effect on muscarinic potentiation of the action potential and does not affect the compound action potential. The ganglia were fixed and sectioned for light and electron microscopy. Ganglia from hamsters that were treated with TMT were markedly different from control ganglia. Multiple vacuolization of the cell bodies with increased numbers of dense bodies, presumably lysosomes, were observed. The nuclei of many cells also acquired an eccentric position. These cellular changes were confirmed with electron microscopy. This investigation indicates that TMT, in addition to its toxic effects on the limbic system, also affects the peripheral nervous system, e.g., the stellate ganglion, inducing both physiological and morphological alterations in these nerve cells. (Supported by EPA Grant # R 809452-01.)

321.5 COMPARISON OF THE ACTION OF TYPES A AND F BOTULINUM TOXIN AT THE RAT NEUROMUSCULAR JUNCTION. L.C. Sellin, J.A. Kauffman*, J.F. Way, Jr.* and L.S. Siegel*. Pathology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701

The clinical features of foodborne botulism suggest that the severity of the disease is dependent on the type of botulinum toxin present (Hughes, J.M. et al., Ann. Intern. Med. 95:442, 1981). Purified type A toxin has been shown to be about 10X more toxic per unit protein than purified type F after intraperitoneal (i.p.) injection in mice (see Bernheimer, A.W., Ed., Perspectives in Toxinology, J. Wiley & Sons, New York, p. 89, 1977). Therefore, we compared the action of these types of botulinum toxin at the rat neuromuscular junction.

Blockade of neuromuscular transmission was produced in the lower hind limb of the rat by local, subcutaneous (s.c.) injection of types A or F botulinum toxin. At various times after injection, the extensor digitorum longus (edl) nerve-muscle preparation was examined for toxin-induced alterations in single twitch and tetanic tension (in situ) or spontaneous and nerve stimulus-evoked quantal transmitter release (in vitro). Doses of toxin greater than 50 mouse i.p. LD_{50} for type A and 10,000 LD_{50} for type F were either severely debilitating or fatal after s.c. injection in rats. Muscles receiving 20 LD_{50} of type A were paralyzed up to and including 7 days after injection. Muscles treated with 8300 LD_{50} of type F were paralyzed at 1 and 3 days, but twitched in response to nerve stimulation by 5 days after injection. Type A toxin reduced the frequency of minature end-plate potentials to a greater extent and for a longer period of time than type F. In cases where quantal content of end-plate potentials (e.p.p.s) was similarly reduced, 3,4-diaminopyridine (3,4-DAP) was more effective in reversing the paralysis in type A than type F-treated preparations. In contrast to type A, 3,4-DAP produced asynchonous (multipeaked) e.p.p.s in type F-treated muscles. These results indicate that: (1) more type F toxin is required to produce effects similar to smaller amounts of type A; (2) type F is shorter acting and (3) type F is less effectively antagonized by 3,4-DAP.

Chemical modification of type F toxin was attempted to determine the basis of its lower potency. Treatment of type F toxin with ethyl acetimidate increased its toxicity by 2-4X after i.p. injection into mice. This procedure also increased the duration of paralysis of the edl after s.c. injection in rats. Presumably, the reaction of imidoesters with protein amino groups forms amidines which are stronger bases than their parent amines. Type F has a lower isoelectric point compared to the more toxic and persistent type A toxin (Bernheimer, p. 89). This factor may play an important role in determining the relative potencies of the toxins.

321.PO FAILURE OF SHORT-TERM TREATMENT WITH CYCLOSPORIN-A TO INDUCE * TOLERANCE TO NERVE ALLOGRAFTS. A.A. Zalewski and A.K. Gulati Lab of Neurochemistry, NINCDS, NIH, Bethesda, MD 20205.

Cyclosporin-A (CyA) is an immunosuppressive agent that is effective in preventing the rejection of nerve allografts. However, in contrast to heart and kidney, nerve allografts behave like skin in that they are gradually rejected once CyA therapy is stopped. The failure to induce tolerance to nerve after the cessation of CyA treatment might be due to the fact that the nerves used previously contained both major and minor transplantation antigens. Accordingly, we have now performed a study in which the allogeneic nerve confronted the CyA-treated host with only minor transplantation antigens. Inbred Fischer (FR) and Lewis (LE) rats (male, 200-250 gm) which differ only in minor antigens were used. FR animals served as hosts, and each one received 4 cm long LE nerves bilaterally in the thigh. Some FR recipients went untreated whereas others received 15 mg/kg of CyA daily for various intervals. LE nerves were rejected within 30 days in all untreated FR hosts. The rejected nerves lacked Schwann, vascular, and perineurial cells, and they were infiltrated by mononuclear cells. In contrast, all LE nerves survived in CyA-treated FR hosts from which, at 30 days, only one of the bilateral LE nerves was taken. The surviving LE nerves were not infiltrated by mononuclear cells, and they contained numerous Schwann cells, blood vessels, and a perineurium. In some FR rats CyA was continued for 3 months and, in each of these, the second LE nerve was found to be filled with myelinated axons, some of which reinnervated muscle. On the other hand, when CyA was stopped after 30 days, these hosts gradually rejected their second LE nerve. When these later animals were regrafted, those which did not receive CyA rejected new LE nerves (in an accelerated second-set manner) whereas others, in which CyA was restarted, accepted them. These results demonstrated that a short-term course of CyA does induce tolerance to nerve allografts bearing only minor antigens; prolonged survival requires continuous CyA treatment. We conclude that CyA, when given continuously, is a potent immunosuppressive agent for nerve allografts, even in sensitized hosts.

321.PO ROLE OF MOLECULAR FORMS OF ACETYLCHOLINESTERASE IN ORGANOPHOSPHATE INDUCED MUSCLE NECROSIS. W-D. Dettbarn, Vanderbilt Univ. Sch. of Med., Dept. of Pharmacology, Nashville, TN 37232

Organophosphorus inhibitors of acetylcholinesterase (AChE) activity in concentrations that cause cholinergic symptoms induced a progressive, dose-related necrosis in rat skeletal muscle fiber. The severity of the myopathy depended on a critical decrease and duration of AChE inhibition. The necrotic muscle fibers are *repaired within one week. Examination of muscle fibers 2 and 3 weeks after a single injection* showed a large number of ragged red fibers in the diaphragm and soleus muscle. Following inhibition, the 4S molecular form of AChE showed the fastest rate of recovery as compared with the 10S, 12S, and 16S forms. AChE of all muscles, after exposure to the inhibitors, showed slow recovery during the first two days. Based on the reappearance of AChE, and assuming first order kinetics, halftime recovery values in days were 5 days for the soleus and 9 days for the extensor digitorum longus (EDL) and diaphragm.

Since in rat muscle the 16S AChE is selectively restricted to the endplate where it may present the physiologically active molecule that regulates neuromuscular transmission by hydrolyzing acetylcholine (ACh), we examined whether inhibition of this enzyme form alone is the major cause of the muscle necrosis. Animals were treated with quaternary phospholine [diethyl-S-(2-trimethyl ammoniumethyl) phosphorothioate], an irreversible inhibitor of AChE, which does not penetrate the cell, and therefore mainly inhibits the externally located 16S enzyme. Other rats were treated with the tertiary form of phospholine [diethyl-S-(2-dimethyl aminoethyl) phosphorothioate], which readily penetrates into the interior of the muscle and inhibits external as well as internal localized AChE. Data indicate that muscles from rats treated with quaternary phospholine showed a high incidence of lesions, supporting the view that inhibition of the 16S form is the trigger for the development of the muscle necrosis. Since tertiary phospholine caused a still higher increase in the number of lesions per muscle, the possibility exists that not all of the 16S forms of AChE is accessible to lipid insoluble inhibitors, and that some activity may be found intracellularly.

This work was supported by NIH Grants NINCDS #12438 and EHS #02028, and a grant from the Air Force #82-0310.

321.PO SPONTANEOUS RECOVERY OF TARDIVE DYSKINESIA SYMPTOMS, Chuong C. Huang. Dept. of Psychiatry, The Medical College of Wisconsin, Milwaukee, WI. 53226.

Seven chronic schizophrenic male patients who had had moderate to severe chronic tardive dyskinesia (T.D.) symptoms were followed up for five years. Their ages are from 55 to 70. The rater and tardive dyskinesia rating scale were maintained constant. For various practical reasons the patients were maintained on the moderate doses of antipsychotic medications. To the author's surprise their T.D. symptoms had attenuated 37.5% to 100% within the five year period. The statistical analysis shows that the mean of the original T.D. scores is 5.143, S.D.=0.508 and the mean of the improved T.D. scores is 1.429, S.D.=0.352. The change is statistical significant T=6.000, DF=12, P<0.01. The postsynaptic receptor hypersensitivity to dopamine is believed to be the cause of T.D. symptoms. There are evidences to support this hypothesis. Based on the findings described above, it appears that as long as the patient is physically healthy, the neuronal membrane or the synapses are able to desensitize and readapt themselve after a long period of time, despite of the facts that the patients are still taking the same medications. The author hopes these findings will provide the baseline for the T.D. researchers and give the patients the hope. More importantly it will help the clinicians to plan their maintainous drug therapy strateges for the chronic schizophrenic patients. The drug therapy to the chronic schizophrenic needs reevaluation.

EPILEPSY: MISCELLANY

322.1 QUANTITATIVE EEG TECHNIQUE FOR MEASURING CORTICAL SEIZURE ACTIVITY: EFFECTS OF EXCITATORY AMINO ACID ANALOGUES. R. Zaczek*, A. Markl*, S. Hazzard* and J.T. Coyle. (SPON:L.Tune) Dept of Neuroscience, Dept. of Psychiatry, Div. of Child Psychiatry, Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

Prior studies on the epileptogenic effects of acidic excitatory amino acid (AEAA) analogues have relied primarily on behavioral observations and/or qualitative assessment of the electroencephalogram (EEG). To allow for more precise comparisons of potency of AEAA and for measurement of anticonvulsant interactions, we have developed an inexpensive minicomputer assisted method for assessing the temporal architecture and total seizure activity caused by convulsant agents. Cortical EEG recorded by a Grass polygraph equipped with 7P11 EEG amplifiers was passed through a beta$_2$-band pass filter and a threshhold comparator and stored on disk with an Apple 2+ minicomputer programmed to record spikes per 3 minute time-bin. The amplitude was adjusted to 50 spikes per 0.5 min period with the comparator sensitivity set at 0.5; for recordings, the comparator sensitivity was increased to 1.0. The program provided a print-out of the beta$_2$ spikes above threshold per 3 min time bin and the cummulative number of spikes for the recording period. Intra-cerebral injections of AEAA (1 ul) were accomplished via a guide cannula stereotaxically placed in the dorsal hippocampus one week prior to the recordings. The standard convulsant, pentylenetetrazol(PTZ) injected intraperitonially caused spike activity in a dose-dependent fashion between 50 and 100 mg/Kg; doses in excess of this resulted in death. Notably, the effects of PTZ had a short duration (20-25 min for 100 mg/Kg) and resulted in only one or two episodes of spike activity and seizures. Intrahippocampal injection of Nmethyl-D,L-aspartate (NMDLA) caused a seizure pattern of intermediate duration lasting up to 3.5 hours. The total spike count was linearly related to dose between 25 and 100 nmoles; doses greater than 125 nmoles did not result in greater effects. In the linear range of the NMDA dose-response curve, dose correlated with intensity and frequency of seizure episodes but not duration. Kainic acid (KA) was the most potent convulsant with a threshold at 200 pmol injected in the hippocampus. KA had a biphasic and quite prolonged pattern of seizure activity. The first 1-3 hours was characterized by episodic seizure bursts folowed by a long period (10-15 hours) of cortical hyperactivity. The total number of spikes correlated with dose between 0.2 nmoles to 2.5 nmoles of KA. Co-injection of 150 nmoles of the NMDA receptor antagonist, 2-amino-7-phosphono-heptanoic acid, with 75 nmoles of NMDA reduced total spike count by 54+11% (N=6;p<0.05). Thus, this method allows for the quantitative assessment of cortical seizure activity suitable for pharmacologic studies.

322.2 ELECTROSHOCK-INDUCED MOTOR PARALYSIS: COMPARISON BETWEEN EAR-CLIP AND CORNEAL ELECTRODES. R.A. Browning, C. Advokat, D.K. Nelson* and L. Isaac. Southern Ill. Univ., Sch. of Med., Carbondale, IL 62901 and Dept. of Pharmacol., Univ. of Ill. Coll. of Med., Chicago, IL 60612.

Rats subjected to 5 electroconvulsive (EC) shocks per day through ear-clip electrodes (at 10-15 min intervals) for 5 consecutive days have been shown to exhibit a reversible motor paralysis (Isaac & Advokat, Soc. Neurosci. Abst. 8 957, 1982). In the present study we have extended these findings by comparing the paralytic effects of EC stimuli (60 Hz, AC, 0.1 sec) delivered through ear-clip electrodes with identical stimuli delivered through corneal electrodes. In the first experiment, two groups of male Sprague-Dawley rats (238g) were subjected to an ascending series of electrical stimuli (40,45,50,55 and 60 mA, 10 min intervals) each day for 5 days with group I (N=12) receiving ear-clip stimulation and group II (N=13) receiving corneal stimulation. Seventy-five percent of the rats in group I were affected (paralyzed or killed) by the treatment, while no rats in group II were paralyzed or killed ($p < 0.01$). Moreover, rats subjected to corneal stimulation exhibited mainly face and forelimb clonus in response to each shock, whereas those subjected to ear-clip stimulation frequently displayed a tonic seizure (body flexion with forelimb extension). In order to determine whether tonic seizures (TS) were a prerequisite for motor paralysis, a second experiment was conducted in which two groups received a stimulus sufficient to cause TS in at least 80% of the subjects. All rats received 5 identical stimuli per day (70mA, 60Hz, AC, 0.1 sec, 10 min intervals) for 5 days with group III (N=12) receiving ear-clip stimulation and group IV (N=12) receiving corneal stimulation. Sixty-seven percent of the rats in group III were affected (paralyzed or killed) whereas only 17% of the rats in group IV were affected by this treatment ($P < 0.05$). However, rats in group IV rapidly developed a resistance to the TS as evidenced by a significant ($P < 0.01$) decline in tonic seizure incidence (TSI) on successive shocks each day (TSI: 89% shock 1, 14% shock 2, and 0% thereafter). In comparison, animals in group III developed this resistance more slowly (TSI: 100% shock 1, 69% shock 2, 30% shock 3, 12% shock 4 and 8% shock 5). Thus, although rats in both groups had a similar TSI on the first shock each day, those in group III (ear-clip) still displayed a higher incidence of TS overall 5 shocks than rats in group IV, suggesting a possible relationship between the seizure severity and the incidence of paralysis and/or death. On the other hand, it seems possible that activation of different anatomical structures or a different order of activating the same structures may be more important than the type of seizure in explaining the differences between ear-clip and corneal electrodes.

322.3 EFFECTS OF SELECTIVE DEAFFERENTATION AND GABA-RELATED DRUGS ON BEHAVIOR-DEPENDENT EEG SPIKES AND NEURONAL BURSTS IN NORMAL HIPPOCAMPUS. S.S. Suzuki and G.K. Smith. Dept. of Psychol., McMaster Univ., Hamilton, Ontario, Canada L8S 4K1.

We have reported (Soc. Neurosci. Abstr., 8: 1017, 1982) that the CA1 region of rat dorsal hippocampus generates EEG spikes (40-100 msec in duration, 0.2-5/sec in frequency) and associated neuronal bursts during behavioral states not accompanied by rhythmical slow EEG activity (RSA) such as awake immobility (AI) and slow wave sleep (SS). Laminar depth profile and evoked potential experiments have shown that the EEG spike (SPK) represents a synchronous excitation (EPSP) of the middle apical dendritic region (radiatum) triggered by burst discharges in CA3 neurons via the Schaffer collateral and commissural fibers.

Effects of large elctrolytic lesions of the medial septal area (MS) and entorhinal cortex (EC) on these behavior-related SPKs were examined. MS lesions, while completely abolishing RSA, did not eliminate SPKs or change their behavioral correlates. They were suppressed during behaviors (walking, etc.) normally associated with RSA. However, SPK frequency was approximately halved after MS lesions. Total bilateral EC lesions in general did not change SPK or RSA unless abnormal activities persisted over the first post-lesion week during which most of the recordings were made. Laminar profiles of SPK in both MS- and EC-lesioned rats showed a normal pattern: positive (small) in oriens, negative (large) in radiatum and polarity reversal around pyramidale.

Diazepam (2mg/kg, i.p.), which enhances GABAergic inhibition, increased fast EEG waves (25-50 c/sec) and greatly reduced SPK frequency (10% of normal rate within 20 min). The GABA antagonist bicuculline (2-8 mg/kg, i.p.) at subconvulsive doses greatly increased (~1000%) the size of population bursts (ripples) associated with larger SPKs, though the frequency of these events was relatively unchanged.

These findings are consistent with the following hypotheses. (1) A reduction in GABA-mediated inhibition produces a physiological condition within the hippocampal circuitry which is favorable for the intrinsic generation of synchronous bursts and associated SPKs. (2) The above condition is realized in the normal rat during states such as AI and SS when ascending brain stem inputs to the hippocampus appear to be weakened. (3) Activation of brain stem afferents concurrent with certain behaviors (walking, paradoxical sleep, etc.) can suppress synchronous bursts and SPKs via either medial septal or entorhinal pathway. (Supported by NSERC and SSHRC)

322.4 PENTYLENETETRAZOL INDUCED SEIZURES PRODUCE AN INCREASED RELEASE OF IR-MET-ENKEPHALIN FROM RAT STRIATUM IN VITRO. M. Zubieta*, O. Vindrola*, M. Asai*, E. Talavera* and G. Linares*. (SPON: P. Huízar). Unidad de Investigaciones Cerebrales del Instituto Nacional de Neurología y Neurocirugía. Insurgentes Sur #3877, 14410 México, D.F. México.

Several pharmacological studies suggest that opioid peptides may be released during an epileptic seizure. We analized the endogenous IR-met-enkephalin (IR-ME) release in striatal slices of rats that showed tonic-clonic generalized seizures after pentylenetetrazol (PTZ) treatment.

Male Long Evans rats (200-250g) were injected i.p. with either saline or 100 mg/kg of PTZ. The PTZ treated animals were sacrificed during the tonic extension. Striata were sliced in two directions at 90º at 300 μm intervals. The resulting slices were suspended in plastic superfusion chambers and were superfused at 1 ml/min. With modified Krebs-Ringer medium, containing 1 mM of phe-ala as enzimatic inhibitor. The release of endogenous IR-ME, induced by low (5 mM) and high (22 mM) K^+ medium, was measured by radioimmunoassay in superfusates recolected during 10 min. An antiserum that showed 0.3% of cross-reactivity with leu-enkephalin was utilized. Values were expressed as ng/g tissue and represent the mean ± S.E.M. of 8 experiments.

The resting release was not modified in the superfusate of PTZ treated animals, but a significative increase occurred with the high K^+ medium (saline: 15 ± 1.3; PTZ: 25 ± 1.5).

GABA, which has been proposed as an inhibitory modulator of enkephalin release in striatum (Osborne H., Arch. Pharmacol., 310: 203, 1980), was perfused at 10^{-4} M, 15 min. previous to potasium ion depolarization. GABA produced a ≃ 63% decrease of enkephalin release in both saline and PTZ treated animals.

These results provide a biochemical evidence that the enkephalins may be released in epileptic seizures and that this effect could be regulated by GABA.

Supported by grant from Instituto Mexicano de Psiquiatría.

322.5 IDENTIFICATION AND QUANTIFICATION OF 1,4 BUTANEDIOL IN BRAIN AND LIVER TISSUES. Steven A. Barker, O. Carter Snead, Chun-Che Liu*, Frederick R. Fish* and R.L. Settine*. University of Alabama in Birmingham GC/MS Center and Department of Pediatrics and The Neurosciences Program, University of Alabama in Birmingham, Birmingham, Alabama.

γ-Hydroxybutyric acid (GHB), occurs naturally in mammalian brain and possesses a number of diverse neuropharmacologic and neurophysiologic properties, including the ability to produce petit mal-like seizures (Snead, Life Sci. 20,1935, 1977). The precursor for GHB in brain is usually considered to be γ-aminobutyric acid (GABA). However, several studies have shown that brain GHB can be significantly elevated by administration of 1,4-butanediol (BD) (Roth and Giarman, Biochem. Pharmacol. 17,735, 1968), thus leading to speculation that BD may also be an endogenous precursor for the biosynthesis of GHB. The problem with this hypothesis has been that BD has yet to be demonstrated as a natural constituent of brain tissue, although its presence in the form of "diol lipids" in rat liver has been suggested (Bergelson et al, Biochem. Biophys. Act. 116,511, 1966).

We have developed a gas chromatographic/mass spectrometric (GC/MS) assay for BD in which BD is derivatized with heptafluorobutyric anhydride. Using this technique we have examined rat brain and rat liver as well as post mortem human brain for the presence of BD.

Our data indicate that BD is indeed a normal component of human brain and rat brain and liver tissues. The highest concentration of BD was observed in the rat liver lipid fraction (314.0 ng/g liver). The concentration of BD in the lipid fraction of human cortex was 14.5 ng/g and 25.5 ng/g in cerebellum. Smaller concentrations of BD were observed in the rat brain lipid fractions and for "free" BD in human brain and rat brain and liver tissues.

These data suggest that BD may well serve as an additional source for GHB in brain and liver.

322.6 **IN VITRO AND IN VIVO EFFECTS OF THE ANTICONVULSANT DRUG, PHENYTOIN, ON CATECHOLAMINE TRANSPORT AND STORAGE IN CHROMAFFIN GRANULES AND SYNAPTIC VESICLES.** **Jean D. Deupree, David A. Downs* and James E. Laposky*, Dept. Pharmacol., Univ. Neb. Med. Ctr., Omaha, NE 68105.**

The anticonvulsant drug, phenytoin, is known to inhibit catecholamine transport into synaptosomes and the metabolism of catecholamines by monoamine oxidase. The significance of these multiple effects of phenytoin depends on whether phenytoin actually alters the storage of catecholamines in nerve endings and consequently the amount of catecholamine that is released from the nerve ending following nerve stimulation. To study this, we examined the in vitro effects of phenytoin (1 μM to 0.4 mM) on the rate of ^{3}H-(-)norepinephrine transport into chromaffin granules isolated from bovine adrenal gland and synaptic vesicles isolated from rat brains. ^{3}H-(-)Norepinephrine transport into both of these vesicles as well as monoamine oxidase activity was inhibited 50% by 0.2 mM phenytoin. Phenytoin appeared to be a competitive inhibitor of catecholamine transport into synaptic vesicles and chromaffin granules and of catecholamine metabolism by monoamine oxidase. In vivo studies were used to determine whether phenytoin altered the amount of catecholamines stored in the synaptic vesicles. For these studies, phenytoin (100 mg/kg) was administered i.p. and the rats were sacrificed by decapitation 75 min later. The brains were quickly removed and the cortex dissected and homogenized in 0.3 M sucrose buffered to pH 7.5 with 1 mM sodium phosphate. Both synaptosomes and synaptic vesicles were carefully isolated and the catecholamine content was measured using HPLC with an electrochemical detector. Under these conditions phenytoin increased the norepinephrine content in synaptic vesicles two fold ($P < 0.001$). In contrast, phenobarbital (<1 mM) had no effect ($P > 0.05$) on catecholamine metabolism, transport or storage in chromaffin granules or synaptic vesicles.

These results suggest that phenytoin competes with catecholamines at multiple sites and that the overall effect of phenytoin is to increase the amount of catecholamine that is released from the nerve endings following nerve stimulation. Since the in vitro effects of phenytoin were noted at toxic rather than therapeutic concentrations of the drug, these effects may be related more to the toxic than the therapeutic actions of the drug. These results may help to explain why phenytoin produces choreoathetoid movement disorders and dystonia in a few individuals. (Supported by Epilepsy Foundation of America).

322.7 MECHANISM OF ACTION OF PHENYTOIN:DIFFERENTIAL EFFECT ON NEURONAL AND GLIAL CELL NA^+,K^+-ATPASE. H. Steve White*, Richard E. Anderson*, John W. Kemp* and Dixon M. Woodbury. Depts. of Pharmacology and Physiology, Univ. of Utah Sch. of Medicine, Salt Lake City, Utah 84132.

Extensive investigation into the mechanism of action of phenytoin (PHT) has demonstrated that this agent decreases inward movement of sodium (Na^+) and calcium (Ca^{++}) ions across biological membranes. Recent studies in this laboratory have demonstrated that chronic administration of PHT (20 mg/kg, ip, bid x 7 days) to adult rats produces a marked gliosis as suggested by an increase in cerebral and cerebellar DNA content and carbonic anhydrase (CA) activity (White, et al., Epilepsia, 24:255, 1983). The effects of PHT on Na^+,K^+-ATPase in relatively pure populations of neuronal (3-day-old rats) and glial (cultured astrocytes) cells, as well as in a mixed population of these cells (adult rats), were examined to localize and define changes in enzyme activity in either neuronal and/or glial cells.

The acute administration of PHT (20 mg/kg) to 3-day-old rats significantly lowered Na^+,K^+-ATPase activity, and, consequently, increased intracellular Na^+, and decreased intracellular K^+ of the cerebral and cerebellar cortices. In adults, the chronic administration (20 mg/kg, ip, qid x 7 days) significantly reduced total cerebral cortex Na^+ content. Although this effect was not accompanied by any significant change in whole homogenate or microsomal Na^+,K^+-ATPase activity, significant increases in myelin (glial product) and marked decreases in synaptosomal (neuronal) activities of this enzyme were observed.

Na^+,K^+-ATPase activity of cultured astrocytes was determined in the presence of increasing K^+ ion concentration (1-20 mM K^+) following acute and chronic exposure to 1 and 10 uM PHT for either 2 hours or 4 days. Both control and PHT treated cells exhibited two distinct peaks in the presence of increasing K^+ ion concentration at 5 and 12.5 mM K^+. These results suggest the existence of two enzymes with different Vmax's and affinities for K^+. Chronic exposure to PHT, activated Na^+,K^+-ATPase at all K^+ concentrations examined in a dose-dependent manner. A similar increase in enzyme activity above control was observed at 1 uM PHT following acute exposure. Acute exposure to 10 uM PHT, however, suppressed enzymic activity at K^+ concentrations greater than 10 mM.

These results support the hypothesis that PHT is primarily an excitatory drug due to the inhibition of neuronal Na^+,K^+-ATPase and that it possesses anticonvulsant activity by virtue of its ability to stimulate predominating inhibitory systems of the mature brain, as well as to produce a reactive gliosis following chronic administration. Thus, by increasing the responsiveness of glial cells to ECF K^+, PHT appears to provide the CNS with an enhanced ability to regulate K^+ ion homeostasis within the brain ECF thereby limiting the spread of seizure activity. Effects on active transport processes of neuronal and glial cells appear to be equally or more important to the mechanism of the anticonvulsant action of PHT than are its effects on Na^+ and Ca^{++} influx. (Supported by NINCDS grant no. NS-15767 and NIGMS grant no. GM-00153).

322.8 HOW EXPERIMENTAL EPILEPSY ALTERS BRAIN DOCOSAHEXAENOATE AND ARACHIDONATE METABOLISM. Nicolas G. Bazan, and T. Sanjeeva Reddy* (SPON: Haydee E.P. Bazan). LSU Eye Center, LSU Medical Center School of Medicine, New Orleans, LA 70112.

Phospholipids of synaptic membranes are enriched in docosahexaenoate. Most of our knowledge of this fatty acid is derived from compositional data and little from metabolic studies. We do know that a single electroconvulsive shock triggers the release of free docosahexaenoic and arachidonic acids in the brain (See Biochim. Biophys. Acta 218:1-10, 1970). Bicuculline-induced status epilepticus also promotes the accumulation of these free fatty acids in brain in both rodents and non-human primates. We have now studied, in bicuculline-induced experimental epilepsy, the obligatory steps that free polyenoic fatty acids must follow in order to be reacylated into membrane phospholipids. The conditions for the assay of the enzymes, docosahexaenoyl-coenzyme A synthetase and arachidonoyl-coenzyme A synthetase, were worked out for microsomal and synaptic membranes. A kinetic study was performed for the activation reaction of docosahexaenoate in synaptic membranes (Km: 5 μm; Vmax: 1.4 nmoles/min/mg protein); and microsomal membranes (Km: 50 μm; and Vmax: 10 nmoles/min/mg protein). For arachidonate, a kinetic study was also performed in synaptic membranes (Km: 11 μm; Vmax: 2.9 nmoles/min/mg protein) and in microsomal membranes (Km: 50 μm; and Vmax: 26.6 nmoles/min/mg protein). These reactions may also play a central role in the regulation of the pool size of free arachidonic acid to be used as a precursor for the synthesis of prostaglandins and lipoxygenase-reaction products. In vivo labeling experiments were also performed by injecting radiolabeled precursors intraventricularly. These studies suggest that in experimental epilepsy, there is a release of free polyenoic acids by phospholipase A_2, as well as a decreased rate of the activation-reesterification reactions. It is not known yet if there are one or two phospholipids A_2 that split arachidonate and docosahexaenoate from phospholipids.

(Supported by a grant from the Esther A. and Joseph Klingenstein Fund, Inc., New York, and from the American Epilepsy Foundation).

322.9 WHAT IS THE DIFFERENCE BETWEEN GRAND MAL AND PETIT MAL DRUGS? G. H. Fromm, C. F. Terrence* and A.S. Chattha*. Dept. of Neurology, Univ. of Pittsburgh, Pittsburgh, PA 15261.

So far, no experimental model has succeeded in fully reproducing human epilepsy in the clinical sense of longtime, recurrent, unpredictable seizures. This lack of a completely satisfactory animal model hampers our ability to reliably screen new anticonvulsant drugs. An alternative approach is to examine the mechanism of action of known antiepileptic drugs on normal neuronal pathways, since anticonvulsants probably act by preventing the spread of the abnormal paroxysmal discharges from the epileptogenic focus to normal neuronal systems. Previous experiments have shown that the various inhibitory and excitatory mechanisms in the trigeminal nucleus of cats are a particularly useful model, since they permit the evaluation of the effect of anticonvulsants on specific inhibitory and excitatory pathways in the CNS.

We have now compared the effects of therapeutic serum concentrations of 1) a drug effective against grand mal and psychomotor seizures (carbamazepine); 2) a drug effective against petit mal and grand mal seizures (valproate); and 3) a drug effective only against petit mal seizures (ethosuximide) in our model. Carbamazepine depressed both afferent and periventricular descending excitatory pathways. It also depressed periventricular inhibition but markedly facilitated segmental inhibition. Valproate depressed periventricular inhibitory and excitatory pathways, but had no effect on afferent excitation and a variable effect on segmental inhibition. Ethosuximide depressed both periventricular and segmental inhibition, but had no effect on periventricular or afferent excitation.

Our results indicate that drugs effective against petit mal seizures specifically depress reticular inhibitory pathways. This would suggest that such seizures are due to paroxysmal discharges in inhibitory pathways resulting in a sudden arrest of ongoing cerebral function. Drugs effective against grand mal seizures depress reticular excitatory pathways, and thus presumably prevent the generalization of paroxysmal activity via the reticular core. Drugs that are also effective against psychomotor seizures additionally facilitate negative feedback mechanisms, thereby limiting the spread of paroxysmal activity from the epileptogenic focus to surrounding neuronal systems.

322.10 EPILEPTIC FOCUS: IS ORGANIC MATRIX NECESSARY? M.S. Myslobodsky, M. Mintz and D. Levin*. Psychobiology Research Unit, Dept. of Psychology, Tel-Aviv University, Ramat Aviv 69978, Israel.

Modern theories of epilepsy relate epileptogenicity to neurochemical and biophysical alterations of the membrane of a single neuron ("epileptogenic neuron") and emphasize the role of functional coupling of individual hyperactive elements creating an epileptogenic constellation ("epileptogenic aggregate"). The ability of some convulsants administered systemically to cause cortical spiking in animals without brain damage seems to support this theory. We have noted, however, that cumulative doses of a steroid derivative, R 5135 (3α-hydroxy-16-imino-5β-17aza-androstan-11-one; Roussel-UCLAF) administered i.p. to Wistar rats caused a prolonged period of unilateral spiking at a dose of 4.0±1.8 mg/kg. An additional dose of the drug (1.9±1.3 mg/kg) was required in order to recruit the other side of the brain. This finding presented a severe anomaly which cannot be explained by the epileptogenic aggregate theory, unless one assumes a profound neurochemical imbalance of the normal brain in rodents.

A hypothesis was tested that asymmetric spiking induced by systemic administration (i.p.) of R 5135 is associated with surgery-related cortical lesions. In 14 animals penetrating (epicortical) silver-ball electrodes were implanted in symmetrical points of the visual cortex. In 11 animals non penetrating (intraosteal) stainless steel electrodes were implanted over one or two sides. The neocortical damage induced by the electrodes was reconstructed on drawings of the appropriate sections. Its area was computed and compared with individual values of the spiking threshold (in mg/kg of R 5135). In 90% of rats, spiking was observed initially over the hemisphere with predominant electrode-inflicted damage. The threshold of spiking was inversely related to the computed area of the lesion ($r[34]=-.66$, $p<.001$). In all rats with epicortical electrodes bilateral spiking could be achieved with additional dose of the convulsant. In 62.5% of the cases where an intraosteal electrode over one hemisphere was yoked with an epicortical electrode over the other one, the non-damaged hemisphere was recruited in bilateral spiking only after administration of a lethal dose of R 5135, or not recruited at all. Lethal dose (16 mg/kg) of R 5135 did not induce spiking in rats with bilateral non-penetrating electrodes. Subconvulsive doses of metrazol (10 mg/kg, i.p.) administered several days later, produced wave-spike discharges on the non-lesioned side. Metrazol given after R 5135, activated wave-spike discharges on the normal side, co-existing with focal spikes on the lesioned side. Hence, whatever neurochemical or biophysical mechanisms of spiking may be discovered in the future, they cannot be divorced from the matrix of structural damage within which they operate.

322.11 GENERALIZED SEIZURES CAN BE PRODUCED FROM A SINGLE ELECTRICAL STIMULATION OF THE INFERIOR COLLICULUS. Thomas J. McCown, Gerald D. Frye, and George R. Breese. Biological Sciences Research Center, UNC School of Medicine, Chapel Hill, NC 27514

Generalized seizures can be ellicited by a single, electrical stimulation of the brainstem in both rabbits and rats (Bergman, Costin and Gutman, Electroenceph. and Clin. Neurophysiol. 15:683, 1963; Chiu and Burham, Neuropharm. 21:159, 1982). In both instances the stimulating electrodes were localized to the brainstem reticular formation, but in rats the threshold current needed for seizures was very high (1000 μA). The present studies identify an area of the inferior colliculus that generates wild running and clonic-tonic motor seizures when electrically stimulated. Rats were implanted with a bipolar electrode in the right inferior colliculus (P100μ, L1.8, V + 1.0, Konig and Klippel, 1963) and allowed seven days for recovery. The threshold test for electrically-induced seizures began at a stimulation current of 100 μA (30 Hz, monophasic square wave, 1.5 msec duration), and the current intensity was increased by 20 μA every 10 sec until wild running was initiated. Continuation of the electrical stimulation over the full 10 sec where wild running occurred led to clonic-tonic seizures. However, the seizure threshold was determined to be the current intensity necessary to produce wild running which outlasted the electrical stimulation by at least 5 sec. Using this criterion, seizures could be electrically-induced at currents as low as 120 μA. Upon repeated testing (every day for one week, or every three days for three weeks) the threshold current for each animal remained stable, neither increasing nor decreasing significantly. Also, the threshold current was reduced when the frequency of the stimulation was increased, up to a maximum at 60 Hz. Stimulation at subthreshold currents evoked no wild running even though the stimulation was continued for 10 min. The wild running threshold was elevated by sodium valporate (200 mg/kg, i.p., 30 min pretreatment), chlordiazepoxide (10 mg/kg, i.p., 30 min pretreatment) but was not elevated by cataleptic doses of haloperidol (1 mg/kg, i.p., 30 min pretreatment). Pentylenetetrazol pretreatment at a subconvulsive dose (15 mg/kg, i.p., 30 min pretreatment) did not alter the wild running threshold but did cause all of the animals to procede into clonus and tonus.

These studies delineate a specific brainstem site from which seizures can be generated. The stable nature of the threshold across time means that this paradigm should prove amenable to the study of seizure mechanisms. (Supported by USPHS grants AA-02334, HD-03110, and grants from the N.C. Alcoholism Research Authority.)

322.12 POSSIBLE ESTROGEN-RELATED CHANGES IN SEIZURE SENSITIVITY. Anne C. Hom*, Pamela H. Feigenbaum*, and Gary G. Buterbaugh. Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, Maryland 21201.

Studies have documented effects of estrogen on neuronal excitability, especially in regard to reproductive behavior, and on changes in levels of transmitter receptor-proteins in the CNS. We have initiated preliminary studies to investigate the relationship between estrogen and seizure sensitivity.

Six Sprague-Dawley rats were bilaterally ovariectomized and compared to 6 intact control rats. Seven days later, all rats began to receive i.p. injections of pentylenetetrazol, 40mg/kg, every other day. Average seizure severity score on the first injection was 1.5 for the control rats and 0.2 for the ovariectomized rats (on a behavioral severity scale for chemically-induced seizures of 0-4). After 4 injections, the control group had reached an average seizure score of 2.8 and the test group 0.6. With succeeding injections, the control group seizure score increased to 3.8 by injection #19. In contrast, the ovariectomized rats remained relatively constant and stayed between 1.8 and 2.7 from injection #5 through #19. This suggests a marked difference in susceptibility to Metrazol^{R} related to ovariectomy, and that a kindling-like phenomenon occurred in the control group that was not observed in the ovariectomized group.

To further test this possibility, a group of 12 animals was ovariectomized and implanted with bipolar electrodes in the right amygdala. Six rats were started immediately on daily subcutaneous injections of estradiol in corn oil, 50mcg/kg. This was sufficient to induce continuous estrus in all 6 animals. Seven days later, the rats were stimulated twice daily with 400 mA, 1-sec. trains of 1-msec., 60 Hz. biphasic, square-wave pulses, spaced at least 4 hours apart. There was no difference in the number of after-discharges (AD's) required to reach two successive Stage V fully-kindled convulsions (on a behavioral scale for electrically-induced seizures of 0-V). However, the rats receiving no estrogen had shorter AD's and accumulated 40% less total AD time than the rats receiving estrogen replacement.

We are currently testing repeated pentylenetetrazol injections in a group of 25 ovariectomized animals, with and without estrogen replacement. After 8 injections, 1 of 12 rats without estrogen replacement has had three successive scale 4 convulsions, compared with 5 of 13 rats with estrogen replacement.

These preliminary results are consistent with postulated influences of estrogen on neuronal excitability. The absence of estrogen in female rats appears to decrease seizure sensitivity. Further work is required to define the role of estrogen in human convulsive disorders.

322.13 INTERACTIONS OF APOMORPHINE WITH LIMBIC SEIZURES. J.A. Conry*, P.F. Moon*, E.W. Lothman, Dept. Neurology, Wash. Univ. Med. School, St. Louis, MO 63110.

In order to investigate whether dopamine (DA) plays a role in the pathophysiology of temporal lobe epilepsy, we studied the interactions of a DA agonist with electrically induced limbic seizures. We employed apomorphine (APO) and produced seizures by means of recurrent tetanic stimuli applied to bipolar electrodes stereotactically implanted in the hippocampus of rats (Soc. Neurosci. Abstr. 8:1018, '82). Sixty to 70 stimuli were elicted every 5 minutes on the first day of stimulation (Day 1) and 16 seizures at 30 minute intervals on subsequent days.

To investigate the effect of APO on limbic seizures, the 30 minute interval stimuli were delivered on 4 consecutive days to two groups of animals. Two days of drug administration (Days 3 and 4 in group 1 and Days 15 and 16 in group 2) separated drug-free days (Days 2 and 5 and Days 14 and 17 respectively). Each seizure was assessed by measuring the duration of the corresponding afterdischarge (ADD) and behavioral seizure score (SS). For each day the intensity of responses in the first block of 8 stimuli was compared to that of the second 8 stimuli using rank sum (SS) and t-tests (ADD). For both groups the mean ADD were longer with APO whereas mean ADD of the first and second blocks were not significantly different on days without APO. The SS of the second block of stimuli were greater than those of the first in 10 of 34 trials with APO and 2 of 34 trials without APO. There was no difference in the other trials.

In another set of experiments we studied the effect of recurrent limbic seizures on APO-induced stereotypy, measured according to a behavioral rating scale. APO (0.05 and 0.1 mg/kg SQ) was administered on days 0, 2, 3, 5, 7 and 9 to two groups of rats implanted with electrodes. One group of rats were stimulated on Days 1 and 2 (see above) and the other received no stimulation. In the non-stimulated rats, APO induced a consistent dose-dependent stereotyped behavior of exploration, sniffing and chewing. In the stimulated rats the stereotypy was augmented on days 3 and 5 returned to normal by day 9.

We conclude that there are two effects of interactions between recurrent limbic seizures and dopaminergic systems. First, DA agonists have a reversible proconvulsant effect toward limbic seizures, increasing the severity of motor convulsive activity and the duration of electrical discharges. Second, recurrent limbic seizures transiently augment DA dependent behavioral responses. These observations suggest that functional changes in DA pathways may be inherent in the expression of temporal lobe epilepsy and associated behavioral changes.

322.14 ANTIEPILEPTIC DRUGS AND STATE-DEPENDENT LEARNING IN GERBILS. H. Kaplan, J. Majkowski*, J. Nowakowski*, and G. Panarello*. N.Y.S. Institute for Basic Research in Developmental Disabilities, SI,NY 10314.

In adults and children at high risk for development of seizures, antiepileptic drugs are often used in pharmacological prophylaxis. Prevention of seizures must be weighed against the possibility of undesirable side effects such as state-dependent learning (SDL). This occurs when a behavioral response, learned when the subject has been treated with a drug, is performed well when the subject is drugged, but is performed poorly or not at all when the subject is tested without the drug. The following experiment was undertaken to see whether two antiepileptic drugs, carbamazepine(CBZ) and diphenylhydantoin(DPH) result in SDL. Subjects were 24 seizure prone Mongolian gerbils from the IBR/SP strain: 12 experimental animals and their 12 sibling controls. Six experimental animals received CBZ (100 mg/kg body wt) and 6 received DPH (30 mg/kg body wt), both dissolved in water to make a volume of 0.06 to 0.09 cc. Half of the controls in each group received a similar volume of water (H_2O group) and the rest were not treated (NT group). Drugs and water were administered per os daily, starting 2 weeks before training in an 8-arm Olton maze began. The arms of the maze which extend radially from a central start box were baited with small pieces of carrot, and subjects were allowed up to 10 min to enter all arms. An error was counted each time an arm was entered more than once. Animals were tested once a day, 5 days a week, until a criterion of no more than 2 errors during each of 3 consecutive tests (criterion tests) was achieved. Drug and water administration was then stopped and testing discontinued. Ten days later subjects were retested for 3 consecutive days.

There were no significant differences among the groups in number of errors or number of tests to criterion, in latency to leave the central start box, time to complete the test, or number of correct choices before the first error was made. There were also no differences between each group's performance during criterion tests and the post-treatment retests in the last 3 measures. There was a significant difference between number of errors made during the 3 criterion tests and the 3 post-treatment retests ($F= 7.00$; $df= 1, 20$; $p < 0.01$). All groups showed a small increase in number of errors during the retests (NT: +0.7, H_2O:+2.7, CBZ: +3.5, DPH: +3.6) but it was only in the DPH group that the increase was significant ("t" for correlated means = 2.8; df = 5; $p < 0.05$). However, considering the small increase in number of errors during the retests and that all groups showed a similar increase, we conclude that mechanisms other than SDL are responsible for the effect.

322.15 AUTORADIOGRAPHIC DEMONSTRATION OF MISMATCHES IN HIPPOCAMPAL METABOLISM AND BLOODFLOW DURING BICUCULLINE-INDUCED SEIZURES. H.-J. Yan*, R.F. Ackermann, W. Meredith* and J. Engel, Jr. Reed Neurological Research Center, and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Systemic administration of bicuculline, a GABA antagonist, causes seizures. However, there is disagreement concerning the effect of systemic bicuculline on hippocampal metabolism; some investigators (Siesjo and Abdul-Rahman, 1979) have reported increases, others (Ben-Ari et al., 1981) decreases. In the present experiments, local cerebral metabolism and bloodflow were studied autoradiographically, using ^{14}C-2-deoxyglucose (2DG) (Sokoloff et al., 1977) or ^{14}C-iodoantipyrine (IAP) (Sakurada et al., 1978), respectively, in unanesthetized, unparalyzed animals. 'Low' (0.2-0.4mg/kgm) or 'high' (0.5-0.8mg/kgm) bicuculline doses were administered (i.v.). Immediately after bicuculline infusion each low-dose animal had a brief (20-60 sec) seizure, recorded from bipolar hippocampal electrodes. Thereafter, hippocampal EEG consisted of polyspike-and-wave complexes arising from a depressed background. Infusion of 2DG occurred 3-12 min post-bicuculline-infusion. Low-dose 2DG autoradiograms resembled those of saline controls, with only slight, nonsignificant, metabolic-rate increases in hippocampus, and substantia nigra. No 'low-dose' blood flow studies were done. Each 'high-dose' animal had a series of severe tonic-clonic seizures that began during drug infusion and continued throughout the experiment; 2DG infusion occurred 3 min post-drug-infusion. Neocortical metabolism increased by a factor of three (3X) over saline-controls. Hippocampus and amygdaloid complex increased 4X, thalamus and globus pallidus 5X. The greatest increase occurred in the substantia nigra (8X), which together with the globus pallidus, subthalamic nucleus and entopeduncular nucleus formed a longitudinal 'core' of extrapyramidal activation. Bloodflow autoradiograms, obtained under identical conditions, showed uptake patterns that differed conspicuously from 2DG patterns. Substantia nigra IAP uptake was not different from surrounding structures; caudomedial amygdala and striatum/accumbens was greater than surrounding structures. Most strikingly, hippocampal bloodflow was greatly reduced relative to adjacent neocortex and thalamus, barely above that of white matter. These results extend our earlier findings (Ackermann et al., 1982) that seizures can result in apparent mismatches between hippocampal metabolism and bloodflow. Similar mismatches in human temporal lobe epileptics (Dymond and Crandall, 1976) could partly account for their high incidence of hippocampal sclerosis.

Supported by grant #NS-15654 from the Public Health Service, and by DOE contract #DE-AM03-76-SF00012.

322.16 THE EFFECT OF REPEATED SEIZURES ON SUBSEQUENT RESPONSE TO ANTICONVULSANT DRUGS IN THE KINDLING MODEL. J.A. Mace* and W.M. Burnham (SPON: L. Grupp). Dept. of Pharmacology, Univ. of Toronto, Toronto, Ontario M5S 1A8.

Clinical studies have suggested that the occurrence of repeated seizures prior to the initiation of anticonvulsant therapy results in a poor prognosis for drug control. This would imply that it is crucial to start anticonvulsant therapy immediately after seizure onset - particularly in the drug-resistant forms of epilepsy such as complex partial attacks. Previous studies in the "kindling" preparation, a model of complex partial seizures, have failed to reproduce this effect. Pre-treatment with 20 focal seizures does not alter the response of amygdala-focal seizures to anticonvulsants (Burnham and Mace, 1983). The present study was designed to further investigate the effect of pre-treatment seizures on subsequent drug control. A variety of experimental parameters were modified in an attempt to reproduce the effect reported in clinical studies.

Rats of the Royal Victoria hooded strain were implanted with chronic stimulating/recording electrodes aimed at the basolateral amygdala. Before drug testing, subjects received either a set number of pre-treatment seizures (experimental group) or matched handling without stimulation (control group). Anticonvulsant response was then assessed using a standard dose-response paradigm.

Individual experiments included an examination of: 1) the effect of an increased number of pre-treatment seizures (40, 100); 2) the effect of stimulation at a "standard" intensity of 400 uA (previous experiments used an intensity of threshold + 40%), and 3) the effect of pre-treatment repetition on other forms of seizure (cortical focal, amygdala generalized). None of these investigations showed any decrease in drug response after pre-treatment seizures. It is concluded that, at least in the kindling model, repeated seizures do not decrease the chances for subsequent control with anticonvulsant drugs.

(This work was supported by the MRC of Canada Grant #MT 5611. J.M. was supported by an MRC studentship.)

CATECHOLAMINES: DOPAMINE RECEPTORS II

323.1 **POSTNATAL ONTOGENY OF DOPAMINE D2 RECEPTORS IN THE RAT STRIATUM.** **L. Charles Murrin, Dept. of Pharmacology, Univ. of Nebraska Medical Center, Omaha, NE 68105**

Biochemical and pharmacological studies have led to a detailed characterization of dopamine receptors in the past decade. Standard procedures have been developed for these studies and we have applied these to examination of the ontogeny of dopamine D2 receptors in the striatum of the rat. Striata from rat pups 0 to 28 days of age were homogenized in 50 volumes of ice cold 0.05 M Tris buffer, pH 7.4 at 37°. These membrane preparations were centrifuged at 48,000 g and then washed twice by the same procedure. Pellets were frozen at -20°C until use. Pellets were resuspended in 0.05 M Tris buffer plus ions, pH 7.4 at 37°C (Eur. J. Pharmacol. 46: 377, 1977) for assay of binding. ^{3}H-Spiroperidol (^{3}H-SP) was used as ligand at 0.5 nM or from 0.1 nM to 40 nM (18 conc.) in saturation experiments. Incubation was 15 min at 37°C and was terminated by filtration over GF/B filters followed by four 5 ml washes with ice-cold buffer. All assays were run in the presence of 30 nM ketanserin as a serotonin receptor blocker. Non-specific binding was measured in the presence of 1 μM (+)butaclamol. There was an increase in ^{3}H-SP binding from 0 to 28 days, as previously reported (Brain Res. 125: 376, 1977). Analysis of saturation studies using the non-linear least squares regression analysis package, LIGAND, indicated this was due to an increase in receptor number and possibly an increase in affinity. At 7 days simultaneous analysis of four experiments gave a K_D of 1.6 nM and a Bmax of 108 fmole/mg protein. A similar analysis at 21 days gave a K_D of 0.30 nM and a Bmax of 143 fmole/mg protein. Pharmacological studies demonstrated the expected properties of dopamine D2 receptors with antagonists displacing ^{3}H-SP at lower concentrations than agonists while drugs active at other neurotransmitter receptors were relatively ineffective. Initial studies on ion dependence and the effects of guanine nucleotides also indicated strong similarities with adult receptors early after birth.

Supported by BNS-7921105 and Grant #1-827 from the March of Dimes Birth Defects Foundation.

323.2 UP-REGULATED D2 SITES IN RAT STRIATUM MAINTAIN THE RATIO OF HIGH TO LOW AFFINITY STATES. Robert G. MacKenzie and Michael J. Zigmond. Department of Biological Sciences, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Agonist displacement of ^{3}H-antagonist describes a biphasic competition curve where the proportion of receptors induced to the high affinity state (R_H) by a given agonist correlates with the drug's intrinsic activity. Thus, changes in R_H might reflect alterations in tissue responsivity to agonists, providing another mechanism for the regulation of synaptic transmission in addition to well-documented changes in receptor density. In the present study, we investigated whether changes in R_H accompany up-regulation of D2 receptors in rat striatum.

Striatal homogenates were pre-incubated at 37°C for 15 min to remove endogeous catecholamines, washed and resuspended in Kreb's phosphate buffer, pH 7.4. D_2 binding was measured using a saturating concentration of ^{3}H-spiperone (0.3 nM) in the presence of .1 uM cinanserin to occlude 5-HT_2 sites. Non-specific binding was measured in the presence of the DA antagonist (+)-butaclamol (1 uM). Non-linear least-squares regression analysis described (+)-butaclamol displacement of ^{3}H-spiperone as a monophasic curve (K_i, 0.33 ± 0.02 nM) which was unaffected by addition of the GTP analog, 0.1 M Gpp(NH)p. Conversely, ^{3}H-spiperone displacement by the DA agonist n-propylapomorphine (NPA) was biphasic K_H, .10 ± .02 nM; K_L, 3.8 ± 1.0 nM, R_H = 64 ± 4%) and was significantly altered by Gpp(NH)p. In the presence of the GTP analog, displacement was monophasic with a K_i (3.2 ± 0.6 nM) similar to that of the low affinity component obtained in the absence of Gpp(NH)p.

Two procedures were used to induce up-regulation of D2 receptors. First, a unilateral injection of 6-HDA (15 ug) was made into the nigrostriatal bundle and rats were killed 7 mo later. Striatal DA content was reduced to 2% of the control side and the B_{max} of lesioned tissue was increased 44%. However, the displacement curve of NPA/^{3}H-spiperone was not affected by the lesion. Second, intact rats were treated with haloperidol (HAL) (1 mg/kg/day, sc) for 54 days and then killed 5 days later. DA and DOPAC levels were unaffected suggesting that the washout period was sufficient. B_{max} of D2 receptors was increased by 43% in the HAL-treated group but the NPA/^{3}H-spiperone displacement curves again were unchanged. We conclude that decreased dopaminergic neurotransmission, produced either by DA depleting brain lesions or chronic receptor blockade, increases D2 receptor density but neither changes the affinity of the sites for NPA nor alters the ability of NPA to induce D2 receptors to adopt the high-affinity state. (Supported by USPHS grants NS-16359 and MH-30915.)

323.3 DOPAMINE RECEPTORS AND THEIR PHYSIOLOGICAL ENDPOINTS IN STRIATUM AND HYPOTHALAMUS ARE REGULATED OPPOSITELY BY CYCLO(LEU-GLY). K.A. Steece, J.M. Lee, R.F. Ritzmann and J.Z. Fields[2], Dept. of Physiology & Biophysics, Univ. Illinois at Chicago, and Dept. of Pharmacology[2], Chicago Med. Sch., North Chicago, Illinois.

Administration of cyclo(Leu-Gly)(CLG), an analog of Pro-Leu-Gly-NH_2(MIF), affects dopamine mediated physiological responses such as stereotypy and hypothermia. The molecular mechanisms of action of these peptides, however, has not been clearly elucidated. Therefore, in the present series of experiments, the effect of in vivo administration of CLG(8 mg/kg, s.c.) was investigated 5 days following administration of a single dose.

It was found that CLG caused a super-sensitive behavioral response(increased stereotypic sniffing) to apomorphine (0.5 mg/kg i.p.) simultaneously with a leftward shift in the curve for dopamine inhibition of [3H]spiroperidol binding to D-2 dopamine receptors(DA-R) in the striatum. By D-2 DA-R we mean those binding sites (R-HI) labelled only by low levels(50 pM) of [3H]spiroperidol(Kd=39 pM) and displaced by 10^{-4} M dopamine. This displacement curve reveals the existence of two sub-populations of D-2 DA-R (R-HI-hi and R-HI-lo). For dopamine, K-HI-hi=10^{-8} M; K-HI-lo=10^{-5} M. Only changes in R-HI-hi correlated strongly with changes in both brain areas.

On the other hand, the same peptide treatment(CLG) caused a sub-sensitivite physiological response(hypothermia) to apomorphine (4.0 mg/kg, i.p.) concomitant with a rightward shift in the curve for dopamine inhibition of [3H]spiroperidol binding to D-2 DA-R in the hypothalamus.

Thus, although the D-2 DA-R in the striatum and hypothalamus appear to be biochemically similar, the results of the CLG studies suggest that a single, peptidergic, neuromodulatory agent (CLG) can elicit diametrically opposite effects on D-2 DA-R and on the corresponding physiological endpoints in two different brain areas

This observation could be accounted for if either (1) there exists a CLG/MIF receptor and if the receptor-effector complex for the peptides are different in the two areas - that is, if there exist CLG-1 and CLG-2 receptor subtypes(possibly due to different second messengers); or if (2) the D-2 DA-R themselves are different in ways that have not yet been detected and that cause them to be regulated differently by a single kind of intermediate response elicited by CLG. (Supported by BRSG grants RR-5366 to JZF and 82508 to RFR)

323.4 ELECTROCONVULSIVE TREATMENT INDUCES DOPAMINE AUTORECEPTORS SUPERSENSITIVITY IN RAT BRAIN. Reches, A.[1], Wagner, H.R., Barkai, A.I.[2], Alter, E.*, Jackson-Lewis, V., and Fahn, S., Department of Neurology, Hadassah Medical Center, Jerusalem, Israel[1], Department of Neurology and the New York State Psychiatric Institute[2], Columbia University, New York, New York.

Electroconvulsive (ECT) treatment has a beneficial, but transitory, effect on patients with Parkinson's disease (PD). The therapeutic efficacy of ECT on PD may be mediated at the receptor level. To test this possibility, we studied the effect of ECT on DA receptors in rat brain. Since DA receptor supersensitivity has been reported in untreated parkinsonian patients we also used haloperidol to induce supersensitivity. Rats were given saline or haloperidol (2 mg/kg) for 28 days. One half the rats in each group received ECT (8 msec at 60 pulses per second for 1.5 sec, 20-30 mAmp, three times per week). Rats were allowed to recover for five days. Apomorphine (0.25 mg/kg) inhibition of DA synthesis in the presence of GBL and NSD-1015 was used to functionally assess putative presynaptic DA autoreceptor sensitivity. Haloperidol and ECT induced supersensitivity of the presynaptic striatal DA receptors as reflected in enhanced APO inhibitions of DOPA accumulations (HAL = 2.39± 0.39; ECT=2.43±0.02; control=3.83±0.51 ng/mg tissue, respectively). Similar results were obtained in the n. accumbens (HAL=1.65±0.19, ECT=1.56±0.10 and control=2.19±0.23 ng/mg, respectively). In rats unchallenged with apomorphine DOPA levels in the striatum and accumbens were 7.19±0.53 and 3.25±0.23 ng/mg tissue, respectively. Sensitivity in rats receiving combined treatment with both ECT and HAL was not significantly greater than levels obtained in rats receiving only one treatment (1.51±0.16 and 1.11±0.06 ng/mg tissue). Density of striatal dopamine D2 binding sites was assessed using ^{3}H-spiperone (SPIP). Specific binding was defined as the difference in ^{3}H-SPIP bound in the presence and absence of 1 um (+)butaclamol. The maximum density of ^{3}H-SPIP was significantly increased by HAL exposure (HAL=408.8±248; control= 226.1±18.6 fmoles/mg protein). The density of ^{3}H-SPIP binding sites in HAL-treated rats was not affected by ECT.

Supported by grants from the Parkinson's Disease Foundation, the Dystonia Medical Research Foundation, the Norman and Barbara Seiden Foundation and NIH grant MH33690.

323.5 THIORIDAZINE METABOLITES ARE MORE POTENT THAN THIORIDAZINE IN REGULATING NEUROTRANSMITTER RELEASE FROM STRIATAL SLICES. D.M. Niedzwiecki*, L.X. Cubeddu, and R.B. Mailman. Division of Clinical Pharmacology, Departments of Pharmacology and Psychiatry and the Biological Sciences Research Center, University of North Carolina, Chapel Hill, North Carolina 27514.

Dopamine receptors in the striatum are believed to play a role in modulating the release of acetylcholine (Ach) from striatal interneurons (via postsynaptic dopamine receptors), as well as the release of dopamine (DA) (presumably via presynaptic autoreceptors). The electrically evoked overflow of ^{3}H-DA and ^{14}C-Ach from perfused rabbit striatal slices was used to assess the relative functional potencies of thioridazine (THD), a phenothiazine antipsychotic drug, and two of its metabolites (THD-2-sulfoxide and THD-2-sulfone). These two metabolites are known to have antidopaminergic properties in vivo and in vitro, and we have hypothesized that they may play a critical role in actions ascribed to THD. Therefore, these compounds were compared for their ability to affect the electrically induced neurotransmitter efflux mediated by the previously mentioned receptor populations.

At low frequencies of stimulation (0.3 Hz; 120 pulses), neither the parent compound nor the metabolites, at concentrations between 10-1000nM, affected the electrically evoked or spontaneous efflux of ^{3}H-DA or ^{14}C-Ach. Other antipsychotic drugs, such as haloperidol or sulpiride, have also been shown to have negligible effects under similar conditions. Next, the compounds were tested for their ability to antagonize the actions of apomorphine. Apomorphine (30nM) inhibited the efflux of ^{3}H-DA (by 70%) and ^{14}C-Ach (by 53%) under the same stimulation conditions. A 50% attenuation of the effects of apomorphine on dopamine overflow required a THD concentration of ca. 100nM. However, only one-tenth the concentration of either of the two metabolites was sufficient to produce the same antagonism of apomorphine. Moreover, the metabolites were found also to have greater potency in attenuating the effects of apomorphine on release of ^{14}C-Ach. The IC_{50} for THD was found to be ca. 1000nM, whereas the IC_{50} for THD-2-sulfoxide and THD-2-sulfone was 10-30nM.

In summary, these results indicate that these two sulfoxidized metabolites of THD are more potent than the parent compound in antagonizing effects mediated by DA- and Ach-release modulatory receptors. These data are consistent with the hypothesis that some of the biological response to THD may be a consequence of its metabolism. Furthermore, the data suggest that in the rabbit caudate nucleus, in vitro, THD may have greater potency for presynaptic DA autoreceptors than for those DA receptors involved in Ach release. If the conclusions drawn from these experiments are valid for other animal species, metabolism and distribution of THD may be important determinants of the effects of the drug.

323.6 CHRONIC TREATMENT WITH BROMOCRIPTINE AND HALOPERIDOL: EFFECTS ON DOPAMINE AND ACETYLCHOLINE RELEASE MODULATORY RECEPTORS. I.S. Hoffmann*, D.M. Niedzwiecki*, M.K. James* and L.X. Cubeddu. Division of Clinical Pharmacology, Department of Pharmacology, University of North Carolina, Chapel Hill, North Carolina 27514.

Chronic treatment with haloperidol has been shown to increase the density of striatal dopaminergic receptors, and to increase the sensitivity of the behavioral responses to apomorphine. Rabbits receiving a daily subcutaneous injection of 1 mg/kg b.wt. haloperidol or its vehicle, for 28 days, were sacrificed by decapitation 96 hrs after the last injection. The electrical stimulation (0.3 Hz, 120 pulses) evoked release of DA and Ach was monitored, and concentration-effect curves for apomorphine on transmitter release were constructed. Treatment with haloperidol failed to modify the stimulation-evoked release of DA and Ach as well as the amount of 3H and 14C present in the tissues. The inhibitory effects of apomorphine on the evoked release of DA and Ach were potentiated in the haloperidol-treated animals, when compared to the control group. However, greater facilitation of apomorphine inhibition was observed for DA than Ach release.

The effects of subacute treatment with bromocriptine on the release of DA and Ach and on the sensitivity of dopaminergic release modulatory receptors to apomorphine were investigated. Rabbits which received a daily subcutaneous injection of bromocriptine (1.5 or 15 mg/kg b.wt.) or its vehicle for 7 days, were sacrificed by decapitation 3 days after the last injection. Pretreatment with higher doses of bromocriptine reduced the evoked release of DA and Ach by 70% and 55%, respectively; without affecting the content of tissue radioactivity. In addition, in the high dose bromocriptine treated animals, the concentration-effect curves to apomorphine were shifted to the right. 30 nM apomorphine inhibited the evoked-release of DA by 68% and 14%, in vehicle and drug-treated animals, respectively. In addition, the same drug concentration reduced Ach release by 57% in vehicle and by 25% in bromocriptine-treated rabbits.

These results indicate that the dopaminergic receptors which modulate the release of DA and of Ach develop adaptational changes after prolonged receptor activation or inhibition. However, the possibility that bromocriptine has persistent effects on striatal DA receptors 3 days after the last injection cannot be ruled out.

This work was supported by the NIH Division of Research Resources Grant RR05406 and the Burroughs Wellcome Clinical Pharmacology Development Award.

323.7 EFFECTS OF BENZTROPINE, BUPROPION, COCAINE AND NOMIFENSINE ON DOPAMINE AND ACETYLCHOLINE RELEASE FROM THE RABBIT STRIATUM. L.X. Cubeddu, I.S. Hoffmann* and M.K. James*. Division of Clinical Pharmacology, Department of Pharmacology, University of North Carolina, Chapel Hill, North Carolina 27514.

The effects of four structurally unrelated neuronal uptake inhibitors (NUI) on the spontaneous efflux and on the electrically-evoked overflow of 3H-dopamine (DA) and 14C-acetylcholine (ACH) in rabbit striatal slices, were investigated. Benztropine (BZT), bupropion (BUP), cocaine (COC) and nomifensine (NOM) (each at 10 μM), reduced the evoked-overflow of Ach in a frequency-dependent fashion, i.e., greater inhibition was seen at 3 than at 0.3 Hz. These effects were antagonized by 1 μM sulpiride, a DA-receptor antagonist.

The NUI had different effects on DA overflow. At 0.3 Hz, BZT and NOM enhanced DA overflow by 120% and 50%, respectively; whereas COC reduced it by 30%, and BUP had no effect. At 3 Hz, BZT and NOM increased DA overflow by 30% and 3%, and COC and BUP inhibited it by 40 and 30%, respectively. However, when the autoreceptors were blocked by sulpiride, NUI produced a large enhancement of DA overflow. At 0.3 Hz, NOM (10 μM) produced a 3.5 fold increase in DA overflow in the presence of 1 μM sulpiride. These findings indicate that the net effect of NUI on DA overflow depends on the balance of two factors: 1. degree of blockage of DA reuptake (which enhances overflow) and 2. degree of autoreceptor activation (which inhibits overflow). The efficient removal of the released DA by the neuronal uptake pump prevents autoreceptor activation at low frequencies and accounts for the small and variable facilitation of DA and Ach release reported with DA antagonists (in the absence of NUI).

Apomorphine (APO) 30 nM, inhibited the overflow of DA and Ach elicited by electrical stimulation. Greater inhibition was seen at 0.3 than at 3 Hz. Sulpiride enhanced DA and Ach overflow at 3 Hz, but not at 0.3 Hz. NOM 1, 3 and 10 μM antagonized the inhibitory effects of APO on DA and Ach overflow, in a concentration-dependent manner. In summary, these results indicate that changes in the synaptic concentration of DA modify the release of DA and Ach, and the responsiveness of autoreceptors to DA agonists and antagonists. The high synaptic concentrations of DA obtained with NUI and/or high rates of stimulation reduce the release of DA and Ach, and antagonize APO effects on DA and Ach release-modulatory receptors.

This work was supported by the NIH Division of Research Resources Grant RR05406 and the Burroughs Wellcome Clinical Pharmacology Development Fund.

323.8 FREQUENCY DEPENDENT EFFECTS OF MUSCARINIC RECEPTOR STIMULATION ON STRIATAL DOPAMINE AND ACETYLCHOLINE RELEASE. M.K. James* and L.X. Cubeddu. Division of Clinical Pharmacology, Department of Pharmacology, University of North Carolina, Chapel Hill, N.C. 27514.

The effects of muscarinic receptor activation on the electrically evoked overflow of ^{3}H-dopamine (DA) and ^{14}C-acetylcholine (Ach) were investigated using rabbit striatal slices. In these experiments, release was measured in the presence of 1 μM sulpiride and 10 μM hemicholinium to prevent the effects of endogenous DA release and to block choline uptake, respectively. Stimulation (120 pulses) at different frequencies (in the presence of hemicholinium and sulpiride) produced varied amounts of DA release. 2.16% of tissue ^{3}H was released at 0.3 Hz, 2.24% at 3 Hz, and 1.69% at 10 Hz. Ach release showed a greater dependence on stimulation frequency. 14.61% of tissue ^{14}C was released at 0.3 Hz, 5.97% at 3 Hz, and 3.33% at 10 Hz.

Indirect stimulation of muscarinic receptors with physostigmine had frequency-dependent effects on DA and Ach release. Physostigmine (1 μM) produced a slight increase in DA release (10-15%). This effect on DA release was blocked by 1 μM atropine. Physostigmine (1 μM) caused a significant inhibition of Ach release: 25% at 0.3 Hz and 50-60% at 3 and 10 Hz. This inhibition of Ach release by physostigmine was completely reversed by 1 μM atropine. Atropine 1 μM had no effect on DA release. Atropine (1 μM) increased Ach release 13% at 0.3 Hz, 6% at 3 Hz, and 30% at 10 Hz.

Direct stimulation of muscarinic receptors with oxotremorine also had frequency-dependent effects on DA and Ach release. Oxotremorine (0.3, 1.0, 3.0, and 10 μM) produced a slight stimulation (5-15%) of DA release. Atropine 1 μM reversed the effects of 10 μM oxotremorine on DA release. Oxotremorine produced an inhibition of Ach release that was dependent on frequency and dose. Inhibition of Ach release was inversely related to frequency at all the doses tested. 10 μM oxotremorine caused a 45% inhibition at 0.3 Hz, 40% at 3 Hz, and 26% at 10 Hz. Atropine (1 μM) only partially reversed the inhibitory effects of 10 μM oxotremorine. The effects of oxotremorine on Ach release were confirmed in studies measuring the electrically evoked overflow of ^{3}H-Ach.

Higher Ach release at low frequencies of stimulation can be explained by an insufficient synaptic Ach concentration to activate the muscarinic receptors modulating Ach release. High frequency stimulation in turn, could produce a higher synaptic concentration of Ach and activation of muscarinic receptors leading to decreased release. The changes in synaptic Ach concentration due to the alteration of stimulation frequency might explain the frequency-dependent effects of these agonists on Ach release.

This work was supported by the NIH Division of Research Resources Grant RR05406 and the Burroughs Wellcome Clinical Pharmacology Development Award.

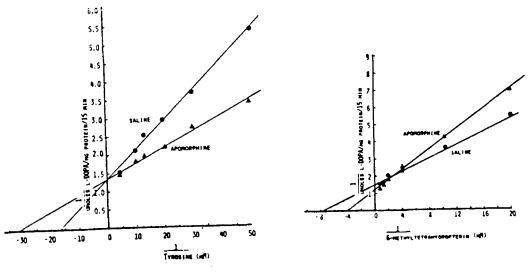
323.9 SELECTIVE DOWN-REGULATION OF DOPAMINERGIC AUTORECEPTORS BY DOPAMINE AGONISTS PRETREATMENTS. ASSESSEMENT BY ENDOGENOUS FAST-PHASE RELEASE OF STRIATAL DOPAMINE. COMPARISON WITH BEHAVIORAL, RECEPTOR BINDING, AND TYROSINE HYDROXYLASE STUDIES. Richard E. Wilcox, John J. Woodward*, Dana M. Vaughn and William H. Riffee. College of Pharmacy, University of Texas, Austin, TX 78712.

Multiple apomorphine (APO) pretreatments (30 mg/kg ip, once per day for 14 days) produce a behavioral supersensitivity to the stereotypic effects of moderate challenge doses of APO (1-4 mg/kg ip) but a behavioral subsensitivity to the inhibition of dextroamphetamine (AMPH)-induced behavioral arousal by low challenge doses of APO (15-80 ug/kg sc). In addition striatal in vitro receptor binding of (3H)-spiperone is unaltered from control values of Kd = 0.128 ± 0.023 nM and Bmax = 0.502 ± 0.008 pmol/mg protein. However in vivo striatal accumulation of l-dopa after decarboxylase inhibition is increased significantly by 20% from control values of 6.6 ± 0.3 ng/mg protein per 30 min. Furthermore, tolerance developes to the ability of in vivo challenge doses of APO and spiperone to decrease and increase, respectively, l-dopa accumulation. Also, the Km of striatal tyrosine hydroxylase for tyrosine is reduced in vitro, while the Km and Vmax for 6-methyltetrahydropterin increase. Taken together, these data suggest that multiple APO pretreatments induce a selective subsensitivity of brain dopamine (DA) autoreceptors including those located on nigrostriatal DA axon terminals (ATARs) without reducing the sensitivity of postsynaptic receptors on nonDA neurons. Such a selective subsensitivity would then be associated with an enhanced release of DA leading to an enhanced APO-induced stereotypic response and a reduced ability of low doses of APO to inhibit the release of DA in conjunction with the locomotor response induced by AMPH.

In order to assess changes in DA release directly a model was needed which is sensitive to ATAR-mediated changes in the release of the newly synthesized catecholamine. In our laboratories we have recently developed a model in which the fast-phase (1 sec.) release of endogenous DA is assessed by HPLC (reverse phase with electrochemical detection). The release is CA^{2+}- and K^{+}-dependent. Under control conditions (60 uM Ca^{2+} and 30 mM vs. 5 mM K^{+} at 1, 3, 5 and 15 sec) the rate of net release declines from 419 pg/sec at 1 sec to 41 pg/sec at 15 sec. In vitro additions of low nM concentrations of APO and of pM concentrations of spiperone are capable of altering fast-phase endogenous DA release from striatum. Also a single in vivo pretreatment with APO results 24 hr later in a behavioral subsensitivity to APO challenge and in a 30% depression of in vitro fast-phase endogenous DA release at 1 sec. These data suggest that the fast-phase endogenous DA release method is sufficiently sensitive to determine directly, changes in DA release in brain regions after chronic DA drug administration. (Supported in part by MH33442 to WHR and REW and UT-BRSG to REW.)

323.10 IN VIVO AND IN VITRO TYROSINE HYDROXYLASE ACTIVITY AFTER DOPAMINE RECEPTOR DOWN-REGULATION. COMPARISON WITH DA and HVA. Dana M. Vaughn, Richard E. Wilcox and William H. Riffee. College of Pharmacy, University of Texas, Austin, TX 78712.

While many recent studies have characterized changes in striatal tyrosine hydroxylase (TH) activity after acute and chronic neuroleptic treatment, little work has been done to establish the effects of multiple doses with dopamine (DA) agonist antiparkinson drugs on the functions of the enzyme. We have recently developed a means of inducing a selective subsensitivity of brain DA autoreceptors (revealed by behavioral and l-dopa accumulation studies) with sparing of postsynaptic receptors from adaptation (demonstrated by binding studies). We have utilized this protocol (involving 14 once daily injections with 30 mg/kg ip of the short-acting DA agonist apomorphine, APO) to evaluate in vivo TH activity in striatum (via l-dopa accumulation after decarboxylase inhibition) and in vitro TH kinetics for tyrosine, 6-methyltetrahydropterin, and DA. Seventy-two hrs after the last APO or saline dose basal l-dopa accumulation was increased by 20% from a control of 6.6 ± 0.3 ng mg^{-1} protein 30 min^{-1}. Tolerance to the ability of acute in vivo challenge with APO or spiperone to decrease or increase, respectively, l-dopa accumulation in striatum occurred after multiple APO pretreatments. Kinetics of in vitro TH for substrate and cofactor are shown below. For tyrosine, Km of TH decreased from 61.3 uM to 32.4 uM after multiple APO treatments, while Vmax was unchanged (0.80 vs. 0.76 uM/mg/15 min). For cofactor analogue, Km of TH increased from 0.13 uM to 0.25 uM after multiple APO treatments and Vmax also increased somewhat (from 0.60 to 0.82 uM/mg/15 min). Neither Ki or Vmax of TH for DA was altered by multiple APO treatments. Comparisons are presented with changes in DOPAC, HVA and 3 MT levels in mice similarly treated. (Supported in part by MH33442 to REW and WHR).



323.11 COVALENT BINDING OF A DOPAMINE AGONIST, APOMORPHINE, IN STRIATUM AFTER CHRONIC ADMINISTRATION. Robert V. Smith, Richard E. Wilcox and Raja B. Velagapudi*. Drug Dynamics Institute and Pharmacology Dept., College of Pharmacy, University of Texas, Austin, TX 78712.

Neurotoxic properties of 6-hydroxydopamine can be attributed to oxidative production of an electrophilic p-quinone which binds covalently to macromolecules. Similarly, the dopamine (DA) agonist apomorphine (APO) may form an ortho-quinone oxidation product in vivo capable of undergoing reaction with protein. Such a putative covalent binding of APO to biological macromolecules may help to explain some of the changs in behavior and in DA release which we have observed following multiple treatments with this aporphine. Initially, we assessed total radioactivity in various organs of mice 24 hr after single vs. 14 daily doses of APO fortified with (3H)-APO. Significant radioactivity (% total dose) was found in GI tract (1.48%), liver (0.38%), kidney (0.11%) and skeletal muscle (0.25% as % dose/g wet wt), after a single APO treatment. After 14 APO doses radioactivity increased to 4.01% in GI tract, 0.99% in liver, 0.33% in kidney and 0.39% in skeletal muscle. Uniform increases in radioactivity accumulation after 14 daily doses vs. the single dose (accumulation ratio 2.6 to 3.0) were found throughout the body except in skeletal muscle (ratio of 1.6). The distribution of radioactivity among different brain regions and plasma after 1 vs. 14 daily APO injections was (as % total dose): plasma (0.56% vs. 1.42% extractable radioactivity), plasma (2.84% vs. 7.51% nonextractable radioactivity), striatum (21.4 ug/g equivalent of albumin, vs. 56.9 ug/g), cerebellum (20.0 ug/g, vs. 57.3 ug/g), and forebrain minus striatum (23.1 ug/g, vs. 69.6 ug/g). Accumulation ratios of 2.6 - 3.0 in plasma and brain indicated uniform increases in radioactivity after multiple APO pretreatments in these tissues as well.

A small percentage (1.7%) of the total radioactivity present in the brain was covalently bound to brain tissues (not removable with multiple washes of trifluoroacetic acid and ethanol) after 14 daily doses of APO. The binding was uniform (as ug/g protein: plasma, 0.68 ± 0.05; striatum, 0.94 ± 0.16; cerebellum, 0.98 ± 0.15; forebrain minus striatum, 1.13 ± 0.14) in the regions of brain examined. Covalently bound radioactivity 24 hr after a single APO dose was below the sensitivity of the assay (< 2 times the background) indicating that covalently bound radioactivity accumulated in the brain after multiple APO doses. Plasma levels of ethyl acetate extractable radioactivity, measured after the single APO dose, declined exponentially from 24 hr to 5 days. The disposition half-life of extractable radioactivity in this phase was 56.8 ± 5.7 hrs which is similar to what we have recently observed in rats (80 hrs). Taken together these data suggest that changes in behavioral and biochemical markers of DA function after chronic APO treatment may be due, in part, to covalently bound APO which, in turn, may be linked to the longer half-life observed in this study. (Supported in part by NS12259 to RVS and MH33442 to REW and W.H. Riffee.)

323.12 PHARMACOGENITICS OF THE NIGROSTRIATAL DOPAMINE SYSTEM: BEHAVIORAL AND BIOCHEMICAL STUDIES OF ELEVEN MOUSE STRAINS. M. Upchurch [1]*, J. Richards [2]*, S. Hall [1]*, T.J. Schallert [1] and R.E. Wilcox [2]. (SPON: W.H. Riffee). [1]Department of Psychology, [2]Division of Pharmacology, University of Texas at Austin, Austin, Texas 78712.

The literature suggests that mice of the recombinant inbred (RI) strains derived from the inbred strains BALB/cByJ and C57BL/6ByJ differ in tyrosine hydroxylase (TOH) activity in the substantia nigra and the striatum and that these differences may reflect a variation in the number of midbrain dopamine neurons and in their degree of branching at their target organs, or in the size of the target organs themselves. We tested animals of the RI and progenitor strains, and mice of the BALB/cJ, CBA/J, and CD-1 strains, for responsiveness to a dopamine antagonist, haloperidol and for performance in undrugged tests of dopamine-mediated behavior. We used four tests of catalepsy to measure responsiveness to haloperidol: a bar catalepsy test, two measures of open field akinesia (latency to move two paws off an index card and latency to move four paws off an index card), and a clinging catalepsy test. For undrugged dopamine-related behaviors we measured exploration of a holeboard apparatus and shredding of a piece of paper (oro-facial stereotype). We compared our behavioral results to the biochemical measures obtained by Vadasz et al. (Brain Res., 234, 1, 1982) and found that exploratory behavior and undrugged oro-facial sterotypy appeared to be related to TOH activity; however, catalepsy varied independently of TOH activity (see below). These results were compared with sterotypy scores after treatment with a dopamine agonist and with in vivo accumulation of l-dopa in the striatum after dopa decarboxylase inhibition. (Supported in part by MH33442 to WHR and REW).

	Substantia nigra		Striatum	
	r	p	r	p
Bar catalepsy	.276	.220	.003	.496
Index card-2 paws	.309	.192	.031	.466
Index card-4 paws	.331	.175	-.030	.467
Clinging catalepsy	-.053	.442	.035	.462
Holeboard 0-5 min	.028	.469	.394	.181
Holeboard 5-10 min	.263	.232	.407	.121
Holeboard 10-15 min	.692	.013	.543	.052
Holeboard 0-15 min	.370	.146	.517	.063
Paper shredding	.567	.044	.272	.223

323.13 NIGROSTRIATAL DOPAMINERGIC CONTROL OF MOVEMENT INITIATION. RELATIONSHIP OF REACTIVE CAPACITY TO (3H)-SPIPERONE BINDING AND DOPAMINE VS. METABOLITES IN NORMAL, DRUG-TREATED AND BRAIN LESIONED RATS. Waneen E. Spirduso[1], Pricilla Gilliam[1], and Richard E. Wilcox[2]. Depts.of Motor Control[1] and Pharmacology[2], University of Texas, Austin, TX 78712.

The role of the nigrostriatal (NS) dopamine (DA) system in the control of motor function has been convincingly established in a qualitative way through studies of motor behavior in aged normal individuals and in those persons suffering from Parkinson's diseae. However, a quantitative assessment of the relationship between NS DA functions and motor behavior has proved more difficult, chiefly because of the limitations of most current paradigms for assessing motor processes. Over the last several years we have developed and evaluated a model of a key aspect of motor behavior, movement initiation (MI), using a rodent reactive capacity (RC) task which closely parallels the tasks in man which are so sensitive to the bradykinesia of Parkinsonism. Our studies have demonstrated the following.

(1) Strain differences. Between and within rat strains, animals with good and poor RC performance show high and low striatal in vitro (3H)-spiperone binding, respectively.

(2) Lesion studies. 6-Hydroxydopamine induced lesions of the NS DA pathway resulting in striatal DA depletions as low as 25% produce deficits in RC performance.

(3) Systemic drug studies. Acute systemic administration of DA agonists and antagonists facilitates and inhibits RC performance, respectively, in a dose-dependent manner.

(4) Aging. Exercise in aging slows the declines in RC performance and in (3H)-spiperone binding to the same degree.

In the present experiments we wished to evaluate the dose-response effects of systemic administration of the DA agonist apomorphine (APO) in relation to NS DA parameters. Male Sprague-Dawley rats were trained on the RC task, which involves the rapid (< 200 msec) release of a lever in response to an auditory CS in an operant paradigm with a UCS of shock and a CS-UCS interval of 200 msec. Each rat received 4 days of testing, with 3 weeks separating each test day. On each test day 50 RC trials with a saline injection were followed by 50 trials with one of 3 doses of APO (0 or 1.25, 2.5 or 5.0 mg/kg, ip). APO doses were counterbalanced (within-subjects design). Three weeks after the last test day rats were sacrificed for determination of striatal (3H)-spiperone binding vs. DA and its acid metabolites. Dose-dependent effects of APO on RC performance were found which predicted striatal (3H)-spiperone binding and DA metabolites. (Supported in part by AG02071 to WWS and MH 33442 to REW and W.H. Riffee).

323.14 THE STEREOSPECIFIC BEHAVIOURAL EFFECTS OF THE ENANTIOMERS OF SK & F 38393 AND THEIR INTERACTIONS WITH DOPAMINERGIC BINDING SITES FOR ^{3}H-PIFLUTIXOL AND ^{3}H-SPIPERONE. John L. Waddington, Kathy M. O'Boyle* and Anthony Molloy*. Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, St. Stephen's Green, Dublin 2.

SK & F 38393 is a racemic, benzazepine compound with atypical properties as a dopaminergic agonist. It is a potent stimulator of dopamine-sensitive striatal adenylate cyclase activity with little affinity for the ^{3}H-spiperone binding site. Therefore, in accordance with prevailing nomenclature for dopamine receptors, it has been proposed as a putative D_1 dopaminergic agonist. In functional studies it fails both to influence prolactin secretion and to induce stereotyped behaviour, consistent with the general view that within the CNS any specific role for the D_1 site is as yet unknown. We have now studied the stereospecific behavioural and receptor pharmacology of the resolved *R*-& *S*-enantiomers of SK & F 38393.

While racemic SK & F 38393 (2.5-40.0 mg/kg s.c.) failed to induce stereotypy it promoted non-stereotyped behaviour, especially grooming and sniffing, when assessed using a behavioural check-list. *R*- but not *S*-SK & F 38393 (both 20 mg/kg) increased the prevalence of intense grooming, sniffing, rearing and locomotion. These effects of the *R*-enantiomer were antagonised by 0.1-0.5 mg/kg cis(Z)-Flupenthixol but not by 0.5 mg/kg trans(E)-Flupenthixol.

^{3}H-piflutixol and ^{3}H-spiperone were used as ligands for D_1 and D_2 dopaminergic binding sites respectively. The activity of SK & F 38393 resided predominantly in the *R*-enantiomer, with a prepotent action at the D_1 over the D_2 site (IC50's 810 nM & 33 μM respectively); its *S*-antipode was only weakly active (IC50's > 50 μM & > 100 μM respectively). While some of the stereospecific behavioural effects of SK & F 38393 may reflect a weak affinity for the D_2 dopamine receptor, grooming is not a typical response to D_2 agonists but was neuroleptic-sensitive. A possible association with selectivity for particular dopaminergic binding sites warrents further study.

This work was supported by the MRC of Ireland and RCSI. We thank SK & F for 38393 enantiomers and Lundbeck for ^{3}H-piflutixol and Flupenthixol isomers.

323.15 BEHAVIORAL AND BIOCHEMICAL EFFECTS OF THE DOPAMINE D1 AGONIST SK&F 38393-A. Hassan, M.N.*++, Reches, A., Kuhn, C.++, Jackson-Lewis, V.*, and Fahn, S., Department of Neurology, Columbia University, New York, NY, and Department of Pharmacology, Duke University Medical Center, Durham, NC.++

SK&F 38393-A activates striatal adenylate cyclase and is characteristic of dopamine D1 agonists. To further determine the selectivity of this compound at the D1 receptor, we have evaluated SK&F 38393-A on a variety of behavioral and biochemical indices. In reserpine-treated (5 $mg.kg^{-1}$) male rats, SK&F 38393-A completely reversed reserpine-induced ptosis, hunched posture and hypomotility. Locomotor activity was stimulated in a dose-dependent manner (2-15 $mg.kg^{-1}$) with a peak effect (up to 700% increase) at 1 h and a duration of up to 3 h. Reserpine-induced catalepsy was lowered; stereotypy was not apparent. SK&F 38393-A produced dose-dependent contralateral rotation in rats with 6-OHDA lesion of the substantia nigra with an RD 500 (dose which produced 500 rotations within 2 h) of 2 $mg.kg^{-1}$.

D2 antagonist properties were also noted. SK&F 38393-A did not induce significant changes in striatal DA or dihydroxyphenylacetic acid (DOPAC) concentrations. Flupenthixol, a putative D1 antagonist, significantly increased striatal DOPAC (0.90±0.1 to 2.2±0.4 $ng.mg^{-1}$ tissue). SK&F 38393-A significantly potentiated the flupenthixol-induced increases in DOPAC (2.2±0.4 to 3.7±0.5 $ng.mg^{-1}$ tissue). SK&F 38393-A (10 $mg.kg^{-1}$) increased serum prolactin (29±1.4 to 96±3.0 $ng.ml^{-1}$) with a peak effect at 0.5 h and a duration of 3 h. SK&F 38393-A inhibited ^{3}H-spiperone binding in striatal membrane preparation with an IC_{50} of approximately 10 µm (calculated Ki=2.0 µm). The failure to induce stereotypy, the potentiation of the flupenthixol-induced increase in striatal DOPAC levels, and the elevation of serum prolactin indicate that SK&F 38393-A may also function as an antagonist at striatal D2 binding sites in rat brain.

Supported by a fellowship from the Ministry of Health, Ontario Canada+, and by grants from the Parkinson's Disease Foundation and the Dystonia Medical Research Foundation.

CATACHOLAMINES: DOPAMINE RECEPTORS III

324.1 COMPARATIVE SOLUBILIZATION OF DOPAMINE RECEPTOR FROM DIFFERENT SPECIES BY DETERGENT SALT COMBINATION. J. Ramwani*, and R.K. Mishra* (SPON: M. Pisa). Dept. of Psychiatry and Neurosciences, McMaster Univ., Hamilton, Ontario Canada.

In order to develop a simple and effective method for the solubilization of dopamine (DA) receptors for subsequent purification, the effectiveness of different detergents was tested. The DA receptors from rat, dog, bovine, and human striata were solubilized by a combination of cholic acid: NaCl and the results were compared with other membrane receptor solubilizing agents.

Species	Detergent	%Specific Binding	% Binding Sites Recovered
Bovine	0.25% Cholic Acid + 1M NaCl	85	35.00
	0.5% Cholic acid + 49% Ammonium Sulphate	65	15.00
	1% Digitonin	30	8.15
	1:5 Digitonin:Cholic acid	29	12.20
	0.25% Cholic acid	25	8.00
Canine	0.25% Cholic acid + 1M NaCl	62	25.00
	0.50% Cholic acid + 49% Ammonium Sulphate	45	15.00
	1% Digitonin	35	11.10
Human	0.25% Cholic acid + 1M NaCl	65	26.00
	0.50% Cholic acid + 49% Ammonium Sulphate	57	-
Rat	0.25% Cholic acid + 1M NaCl	62	30.00

$P_2 - P_3$ (mitochondrial-microsomal) preparation was solubilized with different detergents. ^{3}H spiroperidol binding assay was carried out to characterize the dopamine receptor. The cholic acid: NaCl solubilized receptor exhibited similar affinity and specificity as the membrane receptors of all the species tested.

The solubilized receptors by cholic acid: NaCl treatment satisfied the generally accepted criteria for solubilization, namely, nonsedimentation after centrifugation at 2 x 10^{5} x g for 15 hours, passage through the GF/C filters, absence of laminar membrane under electron microscope.

The solubilized receptors were quite stable at 4°C for 48 hours. Freeze thawing had minimal affect (less than 20%) on the receptor binding. In conclusion, cholic acid: NaCl combination yielded better recovery with maximum % specific binding. Further, this method of solubilization is not species dependent.

(Supported by MRC (Canada).

324.2 CHARACTERIZATION OF SOLUBLE RAT STRIATAL DOPAMINE RECEPTOR BINDING SITES. J.Y. Lew* and M. Goldstein. New York University Medical Center, Neurochemistry Laboratories, New York, N.Y. 10016.

We have previously reported the solubilization of dopamine (DA) receptor binding sites from rat striatum (J.Y. Lew, J.C. Fong and M. Goldstein, Eur. J. Pharmacol., 72, 403-405, 1981). In this study, we have compared some properties of (3-{(3-cholamidopropyl) dimethylammonio}-2-hydroxy-1-propanesulfonate) (CHAPSO) solubilized DA receptor binding sites with that of the membrane bound DA receptors. The solubilized DA receptor binding sites adsorb on wheat germ agarose (WGA) column and are eluted from the column with N-acetyl-glucosamine (GlcNAc). It appears that the striatal DA receptor is a glycoprotein which contains exposed GlcNAc groups. The relative potencies of a wide variety of DA antagonists and agonists to displace ^{3}H-spiroperidol (^{3}H-Spi) from the solubilized DA receptor binding sites are similar to those of the membrane bound.

GTP decreases the affinities of DA agonists (e.g., apomorphine, DA), but not of antagonists to displace ^{3}H-Spi binding from the membrane bound and from the solubilized DA receptor binding sites. These studies suggest that the GTP sensitive component of the receptor complex is also solubilized by CHAPSO. Chronic treatment of rats with haloperidol increases the number of ^{3}H-Spi striatal binding sites of the membrane bound and of the solubilized receptor proteins. Thus, the neuroleptic induced supersensitive receptor binding sites are solubilized by CHAPSO. Pretreatment of the striatal membranes with the sulfhydryl (SH) reagent N-ethylmaleimide (NEM) decreases the ^{3}H-Spi binding of the membrane bound and of the solubilized DA receptor binding sites. The blockade of SH groups by NEM reduces the GTP sensitivity. In summary, the results of our studies suggest that manipulations which alter the binding properties of the membrane bound striatal DA receptors exert a similar effect on the CHAPSO solubilized DA receptor binding sites. Supported by Grants NIMH 02717 and NINDS 06801.

324.3 ^{3}H-SPIROPERIDOL BINDING TO RAT FOREBRAIN SECTIONS. 1. QUANTITATIVE ANALYSIS AND DETERMINATION OF LESION EFFECTS. K.A. Neve, C.A. Altar, C.A. Wong, and J.F. Marshall. Dept. of Psychobiology, University of California, Irvine, CA 92717.

We have examined the kinetic and equilibrium characteristics of ^{3}H-spiroperidol binding to coronal sections of the rat neostriatum and basal forebrain. We also used receptor autoradiography to assess the effects on binding of (1) damage to the ascending DA projection, and (2) intrastriatal injections of kainic acid.

Incubation was carried out in coplin jars containing assay buffer with ^{3}H-spiroperidol for 30 m at 37° C. Specific binding was defined with 1 µM (+)-butaclamol. After rinsing, the 20 µm-thick sections were processed with 1 of 2 procedures. For swabbing studies, sections with cerebral cortex removed were wiped from the slide with GF/B filters and binding was determined by liquid scintillation counting. For the autoradiographic procedure, intact sections were dried, then apposed to tritium-sensitive film (LKB) and stored with tritium standards for 2-4 wks.

In swabbing studies, the rate constants for association (k_1) and dissociation (k_{-1}) of 1 nM ^{3}H-spiroperidol to basal forebrain sections were 7.3 X $10^7 M^{-1}m^{-1}$ and 1.4 X $10^{-2}m^{-1}$, respectively. The K_d calculated as k_{-1}/k_1 was 0.19 nM. Saturation analysis with 0.15-5.0 nM ^{3}H-spiroperidol revealed a Hill coefficient of 1.05. The K_d and B_{max} were 0.93 nM and 454 fmoles/mg protein by Scatchard analysis. The displacement of 2 nM ^{3}H-spiroperidol by several drugs was investigated, and the IC_{50} determined from indirect Hill plots. The following values (in nM) were obtained: spiroperidol, 2.6; butaclamol, 8.3; haloperidol, 56; domperidone, 96; apomorphine, 180; ADTN, 4800; and ketanserin, 4800. In the presence of 100 µM Gpp(NH)p, the IC_{50}s for apomorphine and ADTN displacement of ^{3}H-spiroperidol were increased (2.6 and 74 µM, respectively) and the slope of the curves was increased. Densitometric analysis of autoradiographs revealed a K_d and B_{max} of 0.64 nM and 1170 fmoles/mg protein for neostriatal ^{3}H-spiroperidol binding. Injection of 6-OHDA 7 days prior to sacrifice induced a 15% increase in the receptor density. Intrastriatal injection of kainic acid markedly decreased binding.

Several lines of evidence demonstrate that this site is the high-affinity antagonist DA (D-2) receptor. 1) The rank order of potency for competitive inhibitors is appropriate for that site. 2) Agonist displacement of ^{3}H-spiroperidol binding is sensitive to guanine nucleotides. 3) The receptors are located largely on intrinsic striatal neurons, and postsynaptic to DA terminals. Finally, receptor proliferation occurs within the first week after intracerebral 6-OHDA injection, a period during which behavioral supersensitivity develops rapidly.

324.4 ^{3}H-SPIROPERIDOL BINDING TO RAT FOREBRAIN SECTIONS. 2. COMPUTER-ASSISTED IMAGE PROCESSING. C.A. Altar, R.J. Walter Jr.*, K.A. Neve, and J.F. Marshall. Depts. of Psychobiology and Developmental and Cell Biology, University of California, Irvine CA 92717.

A real time digital image processing system is described that allows for rapid acquisition and analysis of autoradiographs generated by the binding of ^{3}H-ligands to rat forebrain sections that are imaged with a video camera. This method is faster than image processing systems that employ a scanning densitometer (Goochee, D., Rasband, W. and Sokoloff, L., Ann. Neurol. 7: 359, 1980; Herkenham, M. and Pert, C., J. Neurosci. 2:1129, 1982; Palacios, J., Niehoff, D., and Kuhar, M., Neurosci. 7:15,1981). The highly interactive nature of this system allows the autoradiographic images of different brain sections labeled under different conditions to be superimposed and subtracted in real time while being observed on the computer monitor. For example, when an image of a tissue that shows nonspecific ^{3}H-spiroperidol binding is subtracted from a corresponding image of an adjacent section that shows total drug binding, an image is produced that shows the distribution of specific ^{3}H-spiroperidol binding. This procedure is particularly useful for the analysis of autoradiographs for which the non-specific binding of radioactive ligands is a nontrivial proportion of total binding.

Adjacent coronal sections of the rat forebrain were incubated with ^{3}H-spiroperidol or ^{3}H-spiroperidol plus 1 µM (+)-butaclamol and sections were exposed for 4 wks to tritium-sensitive film to produce autoradiographs of total binding and nonspecific binding, respectively. The image processor, consisting of a De Anza Systems IP5500 image processor and LSI-11/23 minicomputer (Walter R. and Berns M., Proc. Natl. Acad. Sci. USA 78:6297, 1981) was used to analyze the autoradiographs in the following sequence: (1) a 480 x 512 x 8-bit digital image was obtained for each section by digitizing and averaging 128 successive video frames, (2) the gray value of each picture element (pixel) in the image was converted to a linear function of ^{3}H-spiroperidol bound per mg protein using a calibration curve obtained from ^{3}H-containing standards, (3) shading distortion was corrected in each image by dividing the image by a second image of a uniformly illuminated field, (4) image subtraction was performed where appropriate to produce images of specific binding, (5) thresholding and boundary following routines were used to isolate particular brain areas or brain sections from background so that area-specific histograms of drug concentration could be produced, (6) images that show the distribution of percent specific drug binding were prepared by dividing images of specific drug binding by a corresponding image of total binding, (7) visual contrast of processed images was then enhanced by expanding the range of pixel gray values in the image or by using a pseudocolor coding transformation routine.

324.5 QUANTITATIVE AUTORADIOGRAPHY OF ^{3}H-APOMORPHINE AND ^{3}H-SPIROPERIDOL BINDING IN RAT BRAIN. E. Richfield*, Z. Hollingsworth*, A. B. Young and J. B. Penney, Dept. of Neurology, University of Michigan, Ann Arbor, MI 48109.

The classification of dopamine receptor subtypes, their regional localization and their regulation by drugs and brain lesions remain controversial. The technique of quantitative autoradiography recently used to study other receptor types was used to study dopamine receptors in rat brain. The dopamine agonist ^{3}H-apomorphine and the dopamine antagonist ^{3}H-spiroperidol were used and demonstrated the advantages of this technique.

Twenty micron sections of rat brain were thaw-mounted onto glass slides and autoradiography was performed as previously described. Sections were rinsed 2 times for 5 min and then incubated for 60 min in 100-25000 pM ^{3}H-spiroperidol in 170 mM Tris.-HCl (pH 7.7), 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.001% ascorbic acid, 1 µM pargyline and 0.1 µM mianserin. ^{3}H-Apomorphine binding (1-100 nM) was performed using 60 min incubation and 50 mM Tris.HCl (pH 7.5), 120 mM NaCl, 2 mM $CaCl_2$, 0.01% ascorbate and 1 µM pargyline. After incubation sections were rinsed 2 X 5 min for both ligands.

Saturation studies revealed a single ^{3}H-apomorphine site in striatum with a K_D of 2 nM and a B_{max} of 60 fmol/mg tissue. Saturation studies using ^{3}Hspiroperidol revealed two sites. The high affinity site had a K_D of 450 pM and B_{max} of 150 fmol/mg tissue. The high affinity site had the following K_is for various drugs: (+) butaclamol, 14 nM; (-) butaclamol (12 µM); haloperidol, 8 nM; dopamine, 75 nM. Regional distribution of ^{3}H-spiroperidol revealed highest binding in olfactory tubercle, striatum, and hippocampus; intermediate binding in layer IV of frontal cortex and amygdala; and lower binding in substantia nigra. Binding in layer IV of cortex was displaced with the serotonin antagonist mianserin. Regional distribution of ^{3}H-apomorphine revealed highest binding in olfactory tubercle and striatum and lower binding in hippocampus, amygdala and substantia nigra. In addition, regulation of dopamine receptors using guanine nucleotides was demonstrated on film. Both 10 µm GTP and 10 µm Gpp (NH)p decreased ^{3}H-apomorphine binding and decreased dopamine competition of ^{3}H-spiroperidol binding.

The advantage of quantitative autoradiography is its ability to do multiple types of studies including saturation curves, competetive inhibition and nucleotide regulation of both dopamine agonist and antagonist binding on a single normal or lesioned rat brain. This technique will help in classifying and localizing dopamine receptor subtypes in various brain regions and their role in disease states.

Supported by NSF grant BNS-8118765 and USPHS grants NS00420 and NS00464.

324.6 TWO COMPONENTS OF SPIROPERIDOL BINDING IN THE RAT STRIATUM, D. R. Liskowsky and L. T. Potter. Dept. of Pharmacology, Univ. of Miami School of Medicine, Miami, Fla. 33101.

Lesion studies utilizing kainic acid have suggested the presence of GTP-sensitive (intrinsic neurons) and GTP-insensitive (cortico-striate terminals) binding sites for ^{3}H-spiroperidol in rat striatum. Sodium (Na) affects the binding of other compounds (dopamine, lisuride, sulpiride) to these same sites. We have investigated these phenomena further in 20 mM Tris-1 mM $MnCl_2$ buffer using unselected membranes from whole striata.

Spiroperidol dissociated exponentially, and yielded linear Scatchard plots. Competition between the benzamide antagonist, sulpiride, and spiroperidol, in Na, also yielded single isotherm-type data. In contrast, agonist competition with spiroperidol, in buffer ±NaCl ±GppNHp, did not yield simple competition curves. Analyses suggested equal populations of "high" and "low" affinity sites. Na alone diminished all agonist affinities; however its effect was greater on high affinity sites. GppNHp alone reduced the affinity of both sites for apomorphine and TL-99 equally, while for dopamine and LY-141685, GppNHp had a greater effect on low affinity sites. Na + GppNHp reduced the affinity for all four agonists, especially at low affinity sites. Alkaline extraction (which is thought to remove coupling proteins) had the same effect as Na + GppNHp.

Taken together with the results of lesion studies, these data indicate that there are two components to the spiroperidol binding in rat striatum: 1) a low agonist-affinity component sensitive to GppNHp and less sensitive to Na, 2) a high agonist-affinity component with greater Na-dependence and less sensitivity to GppNHp. These components may represent binding to the same receptor protein associated with two different membrane mechanisms. One, sensitive to GppNHp, may be associated with post-synaptic sites on intrinsic neurons, while the other mechanism is associated with pre-synaptic sites on cortico-striate terminals.

Supported by N.I.H. grant HL07188 and the National Parkinson Foundation.

324.7 DIFFERENCES BETWEEN D-2 RECEPTORS LABELLED WITH ^{3}H-SPIROPERIDOL AND HIGH AFFINITY NEUROLEPTIC SITES LABELLED WITH ^{3}H-DOPAMINE. A.A. Hancock and C.L. Marsh*, Merrell Dow Research Center, Cincinnati, OH 45215.

Studies of competition assays of 3H-Spiroperidol (3HS) and 3H-Dopamine (3HD) by dopamine receptor agonists and antagonists were carried out to compare the binding of these agents to receptors in rat striatal membranes. Competition curves were analyzed by non-linear least squares analysis (Hancock et al., Mol. Pharmacol. 16:1-9,1979). Saturation assays showed that 3HS bound to a homogenous population of sites with high affinity (Kd=52 pM, Bmax=194±5 fm/mg protein) comparable to previous reports characterizing D_2 dopamine receptors (D_2R). Dopamine (DA) and apomorphine (APO) discriminated between a high and a low affinity component of D_2R. The dissociation constants of DA for these high and low affinity components of D_2R were 22 nM and 1300 nM and for APO were 1.7 nM and 64 nM. Approximately 50% of the total D_2R were of high agonist affinity (103 ± 10 fm/mg protein).

3HD also bound to a homogenous population of sites for agonists with high affinity (2.3 nM). These sites also had a high affinity for APO (0.8 nM). However, the number of sites labelled by 3HD (71±4 fm/mg protein) was significantly less than the number of D_2R (103 fm/mg protein) from which DA displaced 3HS with a high affinity. Moreover, the affinity of DA for 3HD binding sites (2.3 nM) was significantly greater than its high affinity for D_2R (22 nM).

Competition assays of APO and DA for 3HD binding sites were consistent with a homogenous population of sites. However, antagonist competition curves were heterogeneous, consistent with two sites (high and low affinity binding components). Similar previous reports (Hamblin M. and Creese I., Mol. Pharmacol. 21:44-52, 1982) equated the high affinity antagonist sites to D_2R labelled with 3HS. However, our results demonstrated marked differences in the affinity of most neuroleptics to bind to D_2R labelled with 3HS and the high affinity binding of these drugs to sites labelled with 3HD. For S, itself, the difference in Kd's between the two sites was 8-fold. Other neuroleptics had up to 50-fold differences in their affinities at D_2R and the high affinity neuroleptic component of 3HD binding. In summary: 1) both agonists and antagonists had different affinities for a) D2R and b) sites labelled with 3HD for which antipsychotic drugs also had high affinity, and 2) the number of sites labelled by 3HD was significantly less than the fraction of D_2R for which DA had high affinity. These differences in their characteristics suggested that 3HD binding sites having a high affinity for antipsychotic drugs were not the same entity as the D_2R.

324.8 (^{3}H) SPIROPERIDOL BINDING IN STRIATUM OF RATS INFECTED WITH VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS. E. Bonilla, M. Salazar*, H. Hernández* y P. Rangel*. Instituto de Investigaciones Clínicas, Facultad de Medicina, Universidad del Zulia and INBIOMED-FUNDACITE. Apartado 1151, Maracaibo, Venezuela.

We have previously found that after the inoculation of Venezuelan Equine Encephalomyelitis (VEE) virus to rats a significant decrease in the activities of the enzymes glutamate decarboxylase, tyrosine hydroxylase and choline acetyltransferase is produced in the striatum (Bonilla et al, Brain Research 253:330, 1982). These observations do not permit to conclude whether cells lysis or new enzyme synthesis inhibition and/or enzyme inactivation are responsible for the alterations observed in those enzymes during the acute phase of the infection. In order to get a better insight into the pathogenesis of this viral disease we analyzed the (^{3}H) Spiroperidol binding to its striatal receptors in rats infected with VEE virus.

Sprague-Dawley male rats, weighing 200-300 grs. were inoculated intraperitoneally with 0.3 ml of a suspension containing 100 LD_{50} of the Guajira strain of VEE virus in 0.4% bovine albumin borate-buffered saline solution (BABS), pH 7.4. To control animals we administrated equivalent volumes of BABS. They were killed by decapitation simultaneously with the diseased animals, 6-8 days after the inoculation, when the latter presented evidence of encephalitis. Each brain was quickly removed and the neostriatum dissected out at 4°C and stored at -80°C until analyzed. The striatum was homogenized and the incubation performed according to the procedure of Lee et al (Nature 279: 897, 1978). Concentrations of (^{3}H) Spiroperidol ranging from 0.1 to 3.0 nM (specific activity 35.9 Ci/mmol) were used. To detect nonspecific and specific bindings 1uM of (-)Butaclamol or (+)Butaclamol were added to the incubation medium. The density of receptor sites (Bmax) found in VEE infected rats (342.0 ± 23.6 fmol/mg prot ± S.E.) was lower than that observed in controls (474.3 ± 40.6). The difference was statistically significant at $p < 0.05$. The dissociation constants (Kd) were 0.69 ± 0.06 and 0.85 ± 0.10 for the control and infected rats, respectively. The difference was not significant. Since synaptic membranes bearing (^{3}H) Spiroperidol receptors belong to a class of membranes having the same characteristics as the plasma membranes, our results lend support to the assumption that this virus infection produces alterations in the membrane of striatal neurons. This has been shown to be the case for the neuronal population of cerebral cortex in VEE infected rats (García Tamayo et al, J. Path. 128:87, 1978).

324.9 CHRONIC ADMINISTRATION OF D-AMPHETAMINE INCREASES ^{3}H-SPIROPERIDOL BINDING IN CAT BRAIN. T. Crisp and M.E. Trulson. Dept. of Pharmacol., Marshall Univ. Sch. of Med., Huntington, WV 25701.

Chronic administration of amphetamines results in large, long-lasting depletions of brain dopamine in rats and cats. Previous studies reported that long-term amphetamine treatment produced a decrease in ^{3}H-spiroperidol binding in rat brain. This finding has been attributed to a prolonged amphetamine-induced release of dopamine into the synaptic cleft, resulting in down-regulation of dopamine receptors. Behavioral studies in cats, however, have suggested that supersensitivity occurs to dopamine agonists following chronic amphetamine treatment. Therefore, we examined ^{3}H-spiroperidol binding to dopamine receptors in cat brain following chronic drug treatment. Adult cats were administered either saline or d-amphetamine sulfate twice daily for 10 consecutive days, in doses increasing from 10 mg/kg/day (i.p.) to 30 mg/kg/day. Three days after the final injection, the cats were anesthetized with chloral hydrate (400 mg/kg, i.p.), the brains were removed and dissected into the striatum and limbic forebrain (nucleus accumbens, frontal cortex and cingulate gyrus). The tissues were assayed for ^{3}H-spiroperidol binding (0.1-1.5 nM) using (+)-butaclamol (0.1 µM) in blanks for nonspecific binding. K_D and M_{max} values were determined by Scatchard analysis. The data revealed that the B_{max} was significantly increased in both the striatum (+ 28.4%) and limbic forebrain (+ 35.1%) in chronic amphetamine treated cats, as compared to saline injected controls. There were no significant changes in the KD value for either brain region. A single dose of amphetamine (15 mg/kg, i.p.) produced no significant changes in ^{3}H-spiroperidol binding in either brain region, when assayed 3 days following the injection. These data demonstrate the chronic amphetamine administration to cats results in an increase in the apparent number of brain dopamine receptors, in contrast to the decreased dopamine receptor binding reported in the rat studies. These differences may be due to the exceptionally large depletions of dopamine which occur in cats following this amphetamine treatment regimen, i.e., striatal dopamine was decreased by a mean of 96.3%, and limbic forebrain dopamine by 92.8%, following chronic amphetamine treatment. The increased number of dopamine receptors is consistent with the behavioral supersensitivity which occurs to dopamine agonists following chronic amphetamine treatment in cats.

324.10 DO ^{3}H-APORPHINES BIND TO "APORPHINE RECEPTORS" IN MAMMALIAN BRAIN? Arana GW*, Lamont JS*, Baldessarini RJ, Teicher M, Cohen BM* and Neumeyer JL: Departments of Psychiatry and Neuroscience Program, Harvard Medical School (Mailman Research Center) and Medicinal Chemistry, Northeastern University, Boston and Belmont MA 02178.

Low nM concentrations of dopamine (DA) agonists bind selectively to DA "receptor" sites designated as type D-3. We find that the dihydroxyaminotetralin ^{3}H-ADTN binds selectively to such high-affinity sites with pharmacologic characteristics expected of a DA agonist site at an affinity of 0.9 nM (B_{max} = 0.2 nmol/mg protein); its binding is inhibited competitively and monophasically by a wide range of concentrations of R(-)apomorphine (APO, 0.1 nM to 1.0 mM). In contrast, ^{3}H-APO binds to at least two sites: a higher affinity site kinetically indistinguishable from the D-3 ^{3}H-ADTN site, and a lower affinity site (124 nM, 6.0 nmol/mg). Both are included when 1-5 nM ^{3}H-APO is bound and the "blank" is 10 µM ADTN. The lower affinity site is revealed by coincubation with 0.5 µM ADTN to "mask" the high-affinity site for ^{3}H-APO. It has a similar affinity for many aporphines (mean IC_{50} = 178 nM), but much lower affinities for ADTN, DA or other catecholamines (IC_{50} > 10,000 nM), and virtually none for neuroleptics (IC_{50} ≥ 100,000 nM). When ^{3}H-ADTN is coincubated with 0.5 µM unlabeled DA agonist to occlude D-3 sites, none of the above agents binds with affinity below 100,000 nM. The site thus defined for lower-affinity ^{3}H-APO binding, in contrast to its high-affinity D-3 binding, is not stereoselective with respect to several aporphines and catecholamines. Analogous results are obtained in evaluating the pharmacology of binding of ^{3}H-N-chloroethylnorapomorphine (NCA), a proposed long-acting antagonist of DA receptors. Thus, little protection against irreversible binding of ^{3}H-NCA was provided by DA, ADTN or neuroleptics, while aporphines protected most, and in a non-stereoselective manner. The results suggest that, in contrast to ^{3}H-ADTN, tritiated aporphines may, under certain conditions, bind with moderate affinity to possible "aporphine" sites that seem unrelated to DA receptors. (Supported by MH-47370 and MH-34006).

324.11 EFFECTS OF 3-(-3-HYDROXYPHENYL)-N-n-PROPYL-PIPERIDINE (3-PPP) ENANTIOMERS AT DOPAMINE RECEPTORS. N. G. Bacopoulos, S. K. Koch* and B.K. Koe. Department of Pharmacology and Metabolic Diseases, Pfizer Central Research, Groton CT 06340.

The effects of 3-PPP on central dopamine (DA) receptors in rat brain were investigated by in vivo and in vitro methods. The (+) enantiomer reduced and the (-) enantiomer slightly elevated DA metabolites (DOPAC and HVA) in striatal and mesolimbic brain regions. (+)-3-PPP was more potent than (-)-3-PPP in antagonizing the effect of γ-butyrolactone on DOPA accumulation in the striata of mice treated with NSD-1015. These effects suggest that the presynaptic agonist activity resides primarily in the (+) enantiomer.

The reverse enantiomer selectivity was observed in tests of postsynaptic DA receptor function. (-)-3-PPP was a more potent antagonist of the DA-stimulated adenylate cyclase in rat striatal homogenates than (+)-3-PPP. The (-) enantiomer was also more potent as an inhibitor of the stereospecific binding of 3H-spiroperidol or 3H-DA to rat striatal membranes. We have previously shown that the binding sites of 3H-DA are located on elements postsynaptic to the nigrostriatal tract (Life Siences, 32: 531-540, 1983) and may be in part related to the DA-stimulated adenylate cyclase (European. J. Pharmacol., 87: 353-356, 1983).

In summary these results suggest that the (+) enantiomer of 3-PPP is a selective agonist at presynaptic DA receptors whereas its (-) enantiomer is a weak, but selective antagonist at postsynaptic DA receptors.

324.12 SELECTIVE PERIPHERAL DOPAMINE RECEPTOR BLOCKING ACTIVITY OF L-646,462, AN ANALOG OF CYPROHEPTADINE. M. Williams, G. E. Martin, D. C. Remy,* M. Hichens* and B. V. Clineschmidt. Merck Institute, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486-0004.

L-646,462 [(-)-(3-methyl-2,5-dioxo-1-imidazolidinyl)methyl-5-(1-methyl-4-piperidinylidene)-5H-dibenzo[a,d]cycloheptene-3-carboxylate] possesses high affinity (Ki = 23 nM) for rat caudate [^{3}H]-spiperone binding sites and effectively inhibits apomorphine (APO; 15 µg/kg i.v.) induced emesis in beagles (ID_{50} = 32 µg/kg i.v.). Similar values for the reference antidopaminergic antiemetics, metoclopramide (METO) and domperidone (DOM) were 29 and 4 µg/kg i.v., respectively. In assessing the central/peripheral antidopaminergic selectivity of L-646,462 with that of haloperidol, METO and DOM, two sets of comparisons were made: (1) ability to antagonize APO-induced stereotypy (central) in rats as compared to blockade of APO-induced emesis (peripheral) in beagles and (2) using the same rats, the ability to increase striatal HVA levels (central) and serum prolactin levels (peripheral). The ratios obtained (Table) indicate that L-646,462 is less peripherally selective than DOM but more selective than METO.

	ID_{50}* APO-Stereotypy	ID_{50}* APO-emesis	Ratio Stereotypy/ Emesis	$ED_{250\%}$* HVA	$ED_{600\%}$* Prolactin	Ratio HVA/ Prolactin
Haloperidol	83	9	9.2	14.9	10.5	1.4
Metoclopramide	3750	29	129	334	36	9.4
L-646,462	6100	32	188	6974	49	143
Domperidone	20450	4	5113	4800	3.7	1305

* µg/kg i.v.

In addition, L-646,462 has a duration of antiemetic action equivalent to that of DOM (6-8 hrs), both compounds being longer acting than METO (2-4 hrs). These data indicate that L-646,462 exhibits considerable selectivity as a peripherally acting dopamine antagonist and that the compound may be a useful antiemetic agent used in conjunction with dopamine agonist therapy in Parkinsonism.

324.13 A NEW DA RECEPTOR MODEL

H. Wikström*, D. Sanchez*, B. Andersson*, P. Lindberg*, K. Svensson*, S. Hjorth*, D. Clark and A. Carlsson (SPON: G.E. Martin)

Considerable interest has recently been focused on the selective dopamine (DA) autoreceptor agonist 3-(3-hydroxyphenyl)piperidine (3-PPP). In order to determine the structural requirements for rendering this selectivity, 3-PPP and its N-alkyl analogues were resolved. In addition, a series of monophenolic octahydrobenzo(f)-quinolines were synthesized. These tricyclic analogues of 3-PPP were also resolved.

The compounds were evaluated in relation to their ability to interact with central DA receptors. Biochemical and behavioral experimental methods employed were selected on the basis of their ability to distinguish between central pre- and postsynaptic DA receptor interactions.

The results obtained led to the proposal of a new DA receptor model as an extension of current DA receptor theories. This model will be discussed in relation to previously described centrally active dopaminergic compounds and to new agents designed on the basis of this model.

324.14 A PHOTOAFFINITY PROBE FOR THE BRAIN DOPAMINE RECEPTOR: SYNTHESIS AND CHARACTERIZATION. L. L. Hsu and K. Moroi.* Dept. of Psychiatry and Behav. Sci., Univ. of Texas Medical Branch, Galveston, TX 77550.

We have previously reported on the purification and characterization of a dopamine receptor (DA-R) protein from the rat brain using DA-affinity chromatography and disc gel electrophoresis. In addition to the purified DA-R protein with fast mobility on the gel (R_f=0.67), four other protein bands were consistently eluted by 0.1 mM of either haloperidol (HAL), or apomorphine (APO). These proteins appeared to be related to the DA binding sites although no specific ^{3}H-DA or ^{3}H-spiroperidol(SPD) binding was detected for these proteins. These proteins might have lost their binding properties when they were solubilized and further purified. In order to determine whether or not these proteins are actually DA binding sites in the intact synaptic membranes, an azidoaryl derivative of DA has been synthesized as a photoaffinity labelling probe. This nitroazidophenyl-DA derivative (NAP-DA) is photosensitive and upon irradiation will be covalently attached to the DA-binding sites of the synaptic membranes. Thus the radio-labelled photoaffinity probe will allow us to identify each individual DA-binding sites by either native or SDS gel electrophoresis.

Here we describe the synthesis and characterization of the cold NAP-DA. NAP-DA was synthesized according to the method of Darfler and Marinetti (B.B.R.C. 79:1, 1977). Three hundred sixty µmoles of DA·HCl was allowed to react with 140 µmoles of 4-fluoro-3-nitro-phenylazide in 4 ml of 95% ethanol and 3 ml of 0.233 M Na_2CO_3 at 70°C in the dark under a nitrogen flow for 16 hours. The reaction mixture was evaporated to driness and redissolved in 1.5 ml each of $CHCl_3$ and CH_3OH. The reaction products and reactants were separated by TLC using Silica Gel plates. The TLC plates were developed in $CHCl_3$. Two bright orange colored bands were observed on the TLC plates with R_f values of 0.32 (Compound I) and 0.13 (Compound II). Standard FNPA and DA showed R_f values of 0.89 and 0 respectively. The visualized bands were eluted with CH_3OH and replated on the TLC plates for further purification. Both Compounds I and II showed an azido absorption at 2100 cm^{-1} by I.R. analysis. Only Compound II demonstrated displacement effects on the specific ^{3}H-DA binding to the synaptic membranes. The potency of Compound II in displacing the ^{3}H-DA binding was comparable to that of d-butaclamol. Compound II was much weaker than d-butaclamol in displacing ^{3}H-SPD binding. Therefore Compound II would be a good photoaffinity labelling probe specific for the DA-binding site in the brain synaptic membranes. (This work is supported in part by a grant H-827 from the Robert A. Welch Foundation).

324.15 PHOTOLABELING OF CANINE STRIATAL RECEPTOR SITES BY [^{3}H]-CHLORPROMAZINE. K. Thermos, D.I. Schuster and R.B. Murphy, Department of Chemistry, New York University, New York, N.Y. 10003, and L.P. Wennogle and L.R. Meyerson, Department of CNS Research, American Cyanamid Co., Pearl River, N.Y. 10965.

We have extended our earlier studies involving photoincorporation of [^{3}H]-Chlorpromazine ([^{3}H]-CPZ) into a digitonin-solubilized bovine striatal preparation [BBRC, 106, 1469 (1982)] to photolabeling of native (non-detergent treated) canine striatal homogenates. Dopaminergic binding sites were characterized by binding of [^{3}H]-spiroperidol, which was observed to be saturable (K_d= 0.24 nM; B_{max}= 130 fmoles/mg; 0°C). Similar data were obtained at 23° and 37°C. Maximum irreversible incorporation of [^{3}H]-CPZ into the canine preparation was attained after 30 min of UV irradiation at wavelengths > 300 nm. These conditions did not inactivate receptor sites in the absence of [^{3}H]-CPZ, as assayed by [^{3}H]-spiroperidol binding.

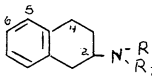
The photolyzed mixture was subjected to SDS-polyacrylamide slab gel electrophoresis with subsequent [^{3}H]-fluorography. Three major bands were observed with approximate molecular masses of 57,000, 34,000 and 32,000 Daltons. When photolysis was carried out in the presence of the D_2 antagonists (+)-butaclamol and (±)-sulpiride, the intensity of these bands was significantly reduced. Similar experiments utilizing the preferential D_1 antagonists cis-flupenthixol as well as a variety of adrenergic, muscarinic and serotinergic receptor ligands as blocking agents will be reported. The relevancy of these data to DA receptor function will be discussed.

324.16 STRUCTURE ACTIVITY RELATIONSHIP OF 2-AMINOTETRALIN DERIVATIVES FOR INTERACTION WITH ^{3}H-CLONIDINE BINDING IN RAT CORTEX. T.K. Chatterjee*, J.P. Long*, J.G. Cannon* and R.K. Bhatnagar. Dept. of Pharmacology, Univ. of Iowa, Iowa City, IA 52242.

In recent years we have synthesized dopamine congeners in order to design compounds selective for specific subpopulation of dopamine receptors. Aminotetralin derivative TL-99 (6,7-dihydroxy-N,N-dimethyl-2-aminotetralin) was suggested to be a selective dopamine autoreceptor agonist (Goodale, D.B., Science, 210, 1141, 1980). However, recent studies questioned the selectivity of TL-99 as a dopamine agonist and suggested its possible interaction with central α_2-adrenoceptor as well (Horn, A.S., Eur. J. Pharmacol., 83, 35, 1982; Pastor, G., ibid 87, 459, 1983). The present study describes structure activity relationship of derivatives of 2-aminotetralin for interaction with ^{3}H-clonidine binding sites in rat cerebral cortex in vitro.

The saturation analysis of ^{3}H-clonidine binding in rat cortex revealed single population of binding sites with K_d and B_{max} values of 2.7 nM and 110 fmoles/mg protein respectively. Assay protocol was essentially the same as described by U'Prichard et al. (Mol. Pharmacol. 16, 47, 1979) except that 2.5 nM ^{3}H-clonidine was used in the binding assay of competing drugs. The binding that was displaced by 10 µM norepinephrine was defined as specific binding. This binding site exhibited characteristics of α_2-adrenoceptor site with the following order of potency expressed as K_i (nM); clonidine 4.5; (-) epinephrine 8.3; phentolamine 16; (-) norepinephrine 22.3; yohimbine 313; apomorphine 367; prazosin 4225; isoproterenol 11350 and serotonin 35000.

The K_i values for hydroxyderivatives of 2-aminotetralin were as follows:



2-Aminotetralin	Code name	Ring OH	R_1	R_2	K_i(nM)
	JOD-173	5,6 di OH	H	H	45.0
	M$_8$	5,6 di OH	H	CH_3	36.2
	TL-259	5,6 di OH	C_2H_5	C_2H_5	42.5
	TL-102	5,6 di OH	C_3H_7	C_3H_7	37.5
	TL-68	none	C_3H_7	C_3H_7	2375.0
	TL-218	6,7 di OH	H	CH_3	4.0
	TL-99	6,7 di OH	CH_3	CH_3	4.2
	TL-196	6,7 di OH	H	C_3H_7	312.5

These data suggest that hydroxyderivatives of aminotetralin also interact with ^{3}H-clonidine binding sites in rat brain cortex and some 6,7-dihydroxyderivatives are as potent as clonidine. In addition, 6,7-dihydroxy substitutions impart greater potency than do the 5,6-dihydroxy substitutions and that N-alkyl substitution either makes no difference or reduces the affinity of these componds for ^{3}H-clonidine binding sites. (Supported in part by NIH grant GM-22365).

324.17 A "USER-FRIENDLY" COMPUTER TECHNIQUE FOR THE NON-LINEAR ANALYSIS OF LIGAND BINDING DATA: ONE AND TWO-SITE MODELS INCLUDING NON-SPECIFIC BINDING. J.E. Lundeen* and J.H. Gordon (SPON: S. Ehrenpreis). Dept Pharmacology, Univ Hlth Scis/The Chicago Med Sch, N. Chicago, IL 60064.

This new method computes the total number of binding sites (B-max) and the dissociation constant (K-d)for each site modeled. In addition the S.E. values for each B-max and K-d, the residuals, the sum of squared error of the residuals, the number of positive and negative residuals, the number of runs in the residuals, and an F-test for comparing the one and two-site models are computed. Also available within the program are user-friendly routines for computing weighted Scatchard and Eadie-Hofstee analyses.

No user supplied initial parameter values are utilized in this program. The user needs only to supply the data set, select the desired model (one or two sites) , and select one of several non-specific binding options. This is accomplished by the use of closed-form equations for the B-max value(s) which provide the means for a direct solution of the B-max value for any given value of K-d. Closed-form equations are not yet incorporated into other binding analysis programs, thus by design they must iterate both the B-max and K-d value(s), while the present method only has to iterate the K-d value(s).

The procedure for a one-site analysis starts with an internal estimate of the K-d value (via Eadie-Hofstee); an error term is then computed for this K-d value. A systematic iteration of the K-d value and its corresponding error term is then used to determine the upper and lower boundaries for the "true" value of K-d. Unlike other non-linear techniques the initial value for K-d need not be accurate. For example if the initial value was 1 X10^6 fold off, the program would determine the boundaries for the "true" K-d within 20 iterations. Once the boundaries have been determined the program rapidly converges on the "true" value for K-d and its corrisponding B-max value.

The two-site analysis begins by generating the one-site K-d value for the data set. The high affinity K-d value has absolute boundaries (i.e. between zero and the one-site K-d value), and the low affinity K-d value has an absolute lower boundary (i.e. the one-site K-d value) and an upper boundary of infinity. This upper limit poses no practical difficulty as a partial differential equation (one of four normal equations) is used to iterate the low affinity K-d in a systematic fashion.

The program can be utilized for the non-linear analysis of both enzyme kinetic and antigen-antibody data. Moreover, the non-linear one-site analysis has been adapted to the TI-59 programable calculator, thus making possible the non-linear analysis of data (without initial estimates of parameter values) on an inexpensive and commonly available laboratory calculator. The complete one and two-site program has been adapted for use on both popular personal computers and a large institutional (i.e. DEC-20) computer.

325.1 ALPHA$_1$- AND ALPHA$_2$-ADRENERGIC BINDING SITES IN HUMAN FRONTAL CORTEX. E. Yablonskaya*, H.R. Wagner, R. Mayeux*, S. Fahn, Departments ot Pharmacology and Neurology, Columbia University, College of Physicians & Surgeons, New York, NY 10032.

Subclasses of alpha-adrenergic receptors have been proposed based on various pharmacologic and physiologic criteria. Radiolabelled ligands have been used to characterize putative alpha$_1$-adrenergic and alpha$_2$-adrenergic binding sites in a variety of tissues. We have used two ot those ligands to identify alpha$_1$- and alpha$_2$-adrenergic binding sites in post mortem tissue from human frontal cortex. Crude synaptic membranes were prepared by homogenization of tissue in iced 50 mM Tris-HCl buffer (pH 7.6) with subsequent centrifugation (40000 x g/10 min). Following two wash steps, pellets were resuspended in the appropriate assay buffer (see below) such that final protein concentrations were 400 mg protein/sample. Alpha$_1$-adrenergic binding sites were labelled with ^{3}H-prazosin (PRAZ); alpha$_2$-sites were labelled with ^{3}H-yohimbine (YOH). For ^{3}H-PRAZ binding membranes were suspended in 50 mM Tris-HCl buffer containing 10 mM $MgCl_2$ (pH 7.6). Incubation buffer for ^{3}H-YOH included 50 mM Tris-HCl, 10 mM $MgSO_4$ and 120 mM NaCl (pH 7.6); final volumes were 1 ml. Specific binding for both assays was defined with 10 uM phentolamine. Incubations were for 30 min at 25°C. Assays were terminated by vacuum filtration over glass fiber filters followed by washing (20 ml). Specific binding of ^{3}H-PRAZ was saturable over a range ot 0.1-2.4 nM. Scatchard analysis was linear with a Bmax=77 fmols/mg protein and a Kd=0.17 nM. The potencies ot unlabelled compounds in displacing specifically bound ^{3}H-prazosin were: PRAZ > phentolamine >YOH. Specific binding of ^{3}H-YOH was saturable over a range of 0.5-20 nM. Scatchard analysis was linear with a Bmax=155 fmols/mg protein and a Kd=3.6 nM. The potencies of unlabelled compounds in displacing specifically bound ^{3}H-yohimbine were: YOH > phentolamine > PRAZ. These data are consistent with the presence of alpha$_1$- and alpha$_2$-adrenergic binding sites in human frontal cortex.

Supported in part by a grant from the Norman and Barbara Seiden Foundation.

325.2 CHARACTERIZATION OF ALPHA-2 ADRENERGIC RECEPTORS IN THE SUBMANDIBULAR GLAND FROM 3-WEEK OLD RATS. D.J. Feller and D.B. Bylund. Department of Pharmacology, University of Missouri, School of Medicine, Columbia, MO 65212.

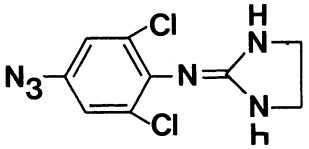
There is no detectable binding of either [^{3}H]yohimbine (YOH) or [^{3}H]p-aminoclonidine (PAC) binding in the adult rat submandibular gland. Reserpine treatment results in the appearance of [^{3}H]clonidine, but not [^{3}H]YOH binding sites. In contrast, both [^{3}H]YOH and [^{3}H]PAC label an alpha-2 adrenergic site in the submandibular from the 3-week old rats. The K_D of [^{3}H]YOH was 5.5 nM which is similar to other rat tissues but 10- to 20-fold higher than human tissues. [^{3}H]PAC (K_D of 2.4 nM) had an affinity which was comparable to most other species. The B_{max} for [^{3}H]YOH and [^{3}H]PAC binding were 205±22 and 256±42 fmol/mg prot, respectively. The overall pharmacology of this site was generally similar to the alpha-2 receptor in other tissues and species.

Drug	K_i (nM)	
	[^{3}H]YOH	[^{3}H]PAC
(-)Epinephrine	222±70	5±1
(-)Norepinephrine	333±99	13±3
(+)Norepinephrine	2637±316	428±116
Yohimbine	13±5	190±26
Phentolamine	3±1	12±3
Prazosin	169±39	10209±1775

However, there were some differences between the rat gland when compared to other species. The K_i of yohimbine at the [^{3}H]PAC labeled site was 190 nM compared to 5 and 7 nM in pig gland and lung, respectively. The ratio of the K_i of yohimbine to the K_i of prazosin at both the [^{3}H]YOH and [^{3}H]PAC sites differed from other species by a factor of 40 to 100. This site apparently was not coupled to adenylate cyclase as in other systems. GTP did not shift the IC_{50} of (-)norepinephrines inhibition of [^{3}H]YOH. GTP induced a dose-dependent increase in the binding of both ^{3}H-ligands. In the pig submandibular and lung GTP causes a decrease in [^{3}H]PAC binding. These results suggest that the sites labeled by [^{3}H]YOH and [^{3}H]PAC in the 3-week old rat gland are atypical alpha-2 adrenergic receptors.

325.3 p-AZIDOCLONIDINE: A POTENTIAL PHOTOAFFINITY LIGAND FOR THE α_2-RECEPTOR. D.C. U'Prichard and P. Ernsberger, Dept. of Pharmacology and Neuroscience Program, Northwestern Univ., Chicago, IL 60611.

p-Azidoclonidine (PAZC) was obtained as a gift from New England Nuclear. ^{1}H and ^{13}C NMR, IR and UV spectra, and high resolution mass spectrum were consistent with the proposed structure:



Competition studies conducted in the absence of light indicated that PAZC may be a selective α_2-agonist. PAZC displayed a high affinity at ^{3}H-p-aminoclonidine (^{3}H-PAC) sites in bovine frontal cortex (IC_{50}= 18±3nM, psuedo-Hill coefficient (n_H) = 0.88±0.03), and a lower affinity at sites labeled by the α_2-antagonist ^{3}H-rauwolscine (IC_{50}= 118±43nM, n_H= 0.54±0.05). PAZC was less potent at α_1-receptors labeled with ^{3}H-prazosin (IC_{50}= 2064±810nM, n_H= 0.88±0.06). PAZC displaced ^{3}H-dihydroalprandlol binding to β-receptors only at high concentrations (IC_{50}= 285±89μM, n_H= 0.61±0.08). In photoaffinity labeling experiments, bovine frontal cortex was homogenized in Tris-HCl buffer (pH 7.7 at 25°C) containing 5mM EDTA, centrifuged at 50,000g for 10 min., resuspended in 100 volumes of Tris-HCl containing 5mM $MgCl_2$, incubated 40 min. at 25°C in the presence or absence of 100nM PAZC or 100nM PAC, and chilled on ice. Half of the samples were then exposed to UV light for 5 min. at 4°C. All samples were then washed 3 times by centrifigation prior to binding assays. In preliminary experiments, pretreatment with 100nM PAZC and UV light decreased the B_{max} of ^{3}H-PAC binding by 40% relative to pretreatment with PAZC in the absence of UV exposure. The K_D of ^{3}H-PAC was unaffected. Pretreatment with 100nM PAZC in the presence of 1μM phentolamine and UV light, or 100nM PAC and UV light, or UV light alone had no effect on ^{3}H-PAC B_{max}. Pretreatment of bovine frontal cortex membranes with PAZC and UV light decreased the B_{max} of ^{3}H-rauwolscine 29%, ^{3}H-prazosin 10%, and ^{3}H-dihydroalprandlol 2%. Protection studies are in progress to examine possible selective interactions of PAZC sith high- and low-affinity states of the α_2-receptor. As a photoaffinity probe, PAZC may be valuable in a labeled form as a tool in the isolation and purification of the α_2-receptor protein.

(P.E. is supported by an NSF predoctoral fellowship)

325.4 GTP DECREASES ANTAGONIST BINDING TO β-ADRENERGIC RECEPTORS OF MOUSE CEREBRAL CORTEX. C.J. Hillard and A.S. Bloom. Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

Most studies of ligand binding to β-adrenergic receptors in peripheral tissues have demonstrated that GTP reduces agonist affinity for the receptor and has no effect on antagonist binding. These findings support a ternary complex model in which a guanine nucleotide binding protein (N-protein) acts as a functional coupler between agonist-occupied receptor and adenylate cyclase. We now report that GTP decreases the binding of both agonists and antagonists to the β-adrenergic receptor of mouse cerebral cortical membranes.

Male ICR mice were sacrificed, cerebral cortical membranes were prepared and binding assays were carried out as described previously (Hillard, C.J. and Bloom, A.S., Brain Res., 235: 370, 1982). ^{3}H-Dihydroalprenolol (^{3}H-DHA), an antagonist, was used as the radioligand in all studies. GTP produced a concentration dependent decrease in the binding of 2.0 nM ^{3}H-DHA with an IC_{50} of 0.3 mM. In the presence of 2.0 mM GTP, ^{3}H-DHA binding was reduced to 27% of control. The equilibrium binding parameters for ^{3}H-DHA were determined in the presence of 0.2 mM GTP. In the absence of GTP, the dissociation constant (K_D) for ^{3}H-DHA was 3.14 ± 0.87 nM and the total binding site density (Bmax) was 92.7 ± 11.3 fmol/mg protein. In the presence of 0.2 mM GTP, the K_D for ^{3}H-DHA was 6.14 ± 1.48 nM while the Bmax was 92.1 ± 12.2 fmol/mg protein. The Hill coefficients were found to be 1.00 and 0.95 in the absence and presence of 0.2 mM GTP, respectively. Therefore, the reduction in ^{3}H-DHA binding produced by GTP was due to a decrease in ^{3}H-DHA affinity.

The effects of 0.2 mM GTP on unlabelled agonist and antagonist competition with ^{3}H-DHA were determined. GTP shifted the competition curve of dl-propranolol to the right, corresponding to an increase in apparent K_i from 4.3 nM to 25.3 nM. The pseudo-Hill coefficients were 0.82 and 0.78 in the absence and presence of GTP, respectively. The competition curve for l-isoproterenol was also shifted to the right in the presence of 0.2 mM GTP. The apparent K_i values were 0.17 μM in the absence of GTP and 1.07 μM in the presence of GTP. The pseudo-Hill coefficients were 0.46 in the absence of GTP and 0.54 in the presence of GTP. Therefore, GTP reduces the affinity of both agonists and antagonists for the β-adrenergic receptor of mouse cerebral cortex.

(Supported by USPHS Grant DA-00124).

325.5 Agonist Interactions with β-Adrenergic Receptors in Rat Brain. J. M. O'Donnell, B.B. Wolfe, and A. Frazer, Depts. of Psychiat. and Pharmacol., Univ. of Penn, and Vet. Admin., Phila., PA, 19104.

The effect of GTP on agonist binding to β-adrenergic receptors on membranes prepared from rat brain was measured by examining agonist displacement of ^{125}I-iodopindolol (IPIN) binding in the absence or presence of GTP. If membranes were prepared in the absence of EDTA in the homogenization medium, then GTP had no effect on l-isoproterenol (ISO) displacement of IPIN. When rat cerebral cortical membranes were prepared with 1mM EDTA in the homogenization medium and 2.5mM $MgCl_2$ in the binding reaction, which was carried out at 37°C, then 250uM GTP increased the Hill coefficient from 0.77±.02 to 0.99±.02 (p<.01) and increased the IC50 for ISO from 88±7nM to 213±21nM (P<.02). The effect of GTP was dose-dependent with an EC50 of about 5uM. The effect of GTP was also temperature dependent. At 17°C, 250uM GTP only increased the Hill coefficient from 0.75±.01 to 0.80±.02 (p<.02) and the IC50 for ISO from 68±3nM to 102±2nM (p<.01). In contrast to displacement curves with ISO, l-propranolol competition curves were steep (Hill coefficient=0.98±.02), and GTP did not affect the displacement due to l-propranolol. Computer-modelling showed that in the absence of GTP, ISO bound to two states of the receptor; GTP converted ISO binding to a single low affinity state. l-Propranolol bound to a single site in the absence or presence of GTP. The effect of GTP on l-epinephrine displacement of IPIN was essentially identical to its effect on ISO displacement. However, even though GTP did increase the Hill coefficient for norepinephrine (NE) from 0.65±.01 to 0.81±.01 (p<.01) and the IC50 from 2.9±0.1uM to 5.4±0.8uM (p<.05), the displacement due to NE in the presence of GTP was still described better by interaction with two sites. It is likely that this reflects the differential affinity of NE for β_1 and β_2 receptors. GTP was the most potent of all the nucleotides tested. 1mM GDP produced effects on ISO displacement of IPIN similar to that seen with 50-250uM GTP. 1mM GppNHp produced only slight shifts in the ISO competition curves and GMP and ATP were inactive. In membranes prepared from rat hippocampus and hypothalamus, ISO displacement curves and GTP effects were qualitatively similar to those observed in cerebral cortex. It appears that agonists, but not antagonists, can stabilize a high affinity ternary complex with the β-adrenergic receptor and the G/F protein in membranes prepared from various regions of the rat brain. The nature of the temperature dependence of the effect of GTP and the low potency of GppNHp suggests differences between guanine nucleotide regulation of β-adrenergic agonist binding in rat cerebral cortex compared to that seen in other systems. (Supported by Research Funds from Veterans Administration and USPHS Grants MH 29094 and MH 14654.)

325.6 THE CHARACTERISTICS OF ADRENERGIC RECEPTORS IN HUMAN PIAL MEMBRANES. Eben Alexander, III*, Allan H. Friedman* and James N. Davis (SPON: B.S. Nashold). Veterans Administration Medical Center and Departments of Surgery (Neurosurgery), Medicine (Neurology), and Pharmacology, Duke University, Durham, NC 27710

Radiolabeled ligands are widely used to elucidate the number and affinity characteristics of specific populations of membrane receptor subtypes. We have studied the adrenergic receptor populations in human pial membranes obtained at autopsy. The vessels obtained ranged in size from small arteries to capillaries. The tissue was homogenized and membranes purified through centrifugation. Nonspecific binding was determined by displacement of [^{3}H]-(-)Dihydroalprenolol (^{3}H-DHA) with 10^{-6} M (±)-propranolol and alpha-adrenergic ligands with 10^{-4} M phentolamine.

^{3}H-DHA in the presence of GTP 10^{-3} M bound to sites (110 fmol/mg protein) with high affinity (K_D = 7.2 X 10^{-9} M), as compared to cortical binding of 37 fmol/mg protein. Displacement of [^{3}H]-DHA from pia was as follows: zinterol>(-)isoproterenol> (-)epinephrine> (-)norepinephrine> (+)isoproterenol. [^{3}H]-DHA displacement data using zinterol and (-)norepinephrine fit best to a 2-site computer model with 60% beta-2 and 40% beta-1. [^{3}H]-Yohimbine bound to a saturable number of pial sites (67 fmol/mg protein) with a high affinity (K_D = 2.3 X 10^{-9} M), compared to cerebral cortical binding of 115 fmol/mg protein. Pial displacement was as follows: WB4101>(-)epinephrine> (-)norepinephrine>prazosin>>(-)isoproterenol. [^{3}H]-Prazosin bound to the membranes with a K_D=1.3 X 10^{-9} M with maximum binding of 39 fmol/mg protein. Displacement of [^{3}H]- prazosin was as follows: prazosin> WB4101 >yohimbine>(-)epinephrine > (-)isoproterenol.

These data show that: (1)there are multiple distinct adrenergic receptors in human pial membranes, (2)there is a slight preponderance of alpha-2 and beta-2 as opposed to alpha-1 and beta-1 receptors, respectively, (3)there are relatively greater numbers of ^{3}H-DHA binding sites in pia than in cortical membranes, as opposed to a greater number of ^{3}H-yohimbine binding sites in cortex than in pia, and (4)the study of radioligand binding in human vessel membranes is feasible in elucidating the number and affinity of different sites. These studies suggest a great heterogeneity in the adrenergic receptor populations of the small blood vessels which play a role in the control of blood flow. This heterogeneity may be found to exist in other areas, such as in cerebral blood vessels.

(Supported by NS 06233)

325.7 CARDIAC β-RECEPTOR BINDING IS REDUCED IN OBESE ZUCKER RATS. S. Bass* and S. Ritter. College of Veterinary Medicine, Washington State University, Pullman, WA 99164-6520.

Recent studies suggest that the function of the sympathetic nervous system is impaired in genetically obese Zucker rats. Obese rats differ from their lean littermates with respect to basal catecholamine levels in both hypothalamus and plasma and are deficient in their ability to elevate plasma catecholamine levels in response to stress (B.E. Levin, et al., Pharmacol. Biochem. Behav. 13:107-113, 1980). Postsynaptic sympathetic mechanisms may also be impaired since adipocytes from brown adipose tissue of obese Zuckers have greatly reduced numbers of β-adrenergic binding sites per cell, compared to lean Zuckers (B.E. Levin, et al., Am. J. Physiol. 243:E217-E224, 1982).

In the present experiment we measured β-adrenergic receptor binding in cardiac membranes of obese (n=6) and lean (n=6) adult female Zucker rats in order to determine whether receptor deficits are present in sympathetically-innervated nonadipose tissue of the obese animals. Cardiac membranes were prepared from whole hearts minus atria and fibrous rings. Using ^{3}H-dihydroalprenolol as the ligand and isoproterenol as the displacing agent in a standard in vitro binding assay, the density of cardiac β-receptors and the apparent K_D of the radioligand were determined by Scatchard analysis. Although the apparent K_D values for binding did not differ between groups, the concentration of cardiac β-receptors was 33% lower in fat rats, compared to the value obtained in lean littermate controls (37.23 ± 3.2 VS 55.55 ± 7.5 fmol/mg protein, respectively, p < .05). Our results suggest that decreased β-adrenergic receptor concentration may be generally present in sympathetically-innervated tissues of obese Zucker rats and support the view that such abnormalities could contribute to the pathogenesis of obesity and its complications.

325.8 KINETICS AND PHARMACOLOGY OF [^{125}I]-IODOHYDROXYBENZYLPINDOLOL BINDING TO THE GLIAL CELL LINE LRM-55. V. Madelian* and W. G. Shain. Center of Labs and Research, NYS Dept. of Health, Albany, NY 12201

Binding of [^{125}I]-Iodohydroxybenzylpindolol ([^{125}I]IHYP) to cultured intact LRM-55 cells shows the presence of beta adrenergic receptors.

The LRM-55 cell line was obtained from a single cell clone of an ethyl nitrosourea-induced spinal cord tumor. Primary pathology described the tumor as a mixed glioma. This cloned cell line exhibits a number of glial cell properties including high-affinity transport of the amino acid neurotransmitters taurine and glutamate [D. L. Martin and W. Shain. J. Biol. Chem. 254: 7076 (1979); R. E. Waniewski and D. L. Martin, Brain Res., in press], anion exchange and transport (E. Wolpaw and D. L. Martin, Trans. Am. Soc. Neurochem. 13: 113 (1982) and synthesis of glial cell growth factor [Kato et al, Proc. Jap. Cancer Association, (1982)].

Binding of [^{125}I]-IHYP is routinely carried out at 37°C in test tubes containing the radioligand and cells freshly scraped from petri plates and suspended in HEPES buffered Hank's saline (pH 7.3). At the end of the incubation, contents of each tube are collected on glass fiber filters, washed and counted for radioactivity. Specific binding is determined as the difference between binding in the absence and presence of 10^{-6}M l-propranolol.

This binding is proportional to cell density, dependent on temperature and ligand concentration, and saturable with time and ligand concentration. At 37°C, specific binding increases rapidly for approximately 12 minutes. It then proceeds at a slower rate and reaches a plateau by 45 minutes. Binding is slower at 20°C and much slower at 4°C. Equilibrium binding studies show specific binding to be saturable with a B_{max} of 28 fmoles/mg protein and a Km of 84 pM. Competetive binding studies in the presence of agonists and antagonists show a rank order of potency characteristic of beta-receptor binding, with propranolol being the most potent inhibitor of binding, followed by alprenolol, isoproterenol, norepinephrine and epinephrine. Binding inhibition is also stereospecific, with l-isomers being 10 to 100 times more potent than their d-counterparts.

325.9 REGULATION OF TYROSINE HYDROXYLASE ACTIVITY BY CELL-TO-CELL INTERACTION. Robert A. Ross, Dept. of Biol. Sci., Fordham University, Bronx, N.Y.

The activities of the catecholamine biosynthetic enzymes in neuronal cells grown in vitro change depending upon the phase of the growth cycle; enzyme activity increasing with increasing cell-to-cell interaction. This mechanism of enzyme regulation has been difficult to study because of the heterogeneity of interactions between cells grown on substrate. In the present study, a culture method has been employed to maintain the cell-to-cell interactions at a stable level in vitro. This was accomplished by growing neuronal cells as cell aggregates in suspension culture.

The human neuroblastoma clonal cell line SK-N-BE(2)-M17, designated BE(2)-M17, was grown either on substrate in 25 cm^2 flasks or in suspension in Erlenmayer flasks in a gyratory water bath shaker. The activity of tyrosine hydroxylase (TH) was assayed at various times after passage. When grown on substrate, TH activity decreased by day 1 and remained reduced until cells approached stationary phase as cell-to-cell interaction increased. Thereafter, TH activity increased rapidly. In contrast, BE(2)-M17 cells grown in suspension formed small aggregates of uniform size (2032 $\pm$ 37 cells/aggregate). TH activity was stable within these aggregates throughout the two week growth period.

Glycoproteins and glycolipids have been suggested as potential mediators of cell recognition and communication. To determine whether sugar moieties on the cell surface play a role in the regulation of TH activity, specific plant lectins were added to the suspension cultures. The effect of aggregate size and TH activity of the neuronal cells on BE(2)-M17 was determined three days after the addition of 100 µg/ml of phytohemaglutinin (PHA), soybean agglutinin (SBA), and wheat germ agglutinin (WGA). While PHA and SBA had no effect on either aggregate size or TH activity, WGA caused a greater than 50% decrease in TH activity within the aggregates. This effect was blocked by the addition of the appropriate haptene. There was no change in the number of cells/aggregate. Thus, WGA, which binds specifically to N-acetyl-D-glucosamine residues on the cell surface, reduced TH activity in aggregates of neuronal cells.

Thus, in the present study, a culture method is used to maintain neuronal cells with stable enzyme levels and cell-to-cell interaction. Blockage of N-acetyl-D-glucosoamine moieties on their surface reduces TH activity within these cells. Thus this sugar may play a role in the regulation of TH activity by cell-to-cell interaction.

(Supported by NIH grant 17738)

325.10 ASSOCIATION OF SEQUESTERED BETA-ADRENERGIC RECEPTORS WITH THE PLASMA MEMBRANE: A NOVEL MECHANISM FOR RECEPTOR DOWN-REGULATION. C. D. Strader* and R. J. Lefkowitz* (SPON: D. R. Sibley). Howard Hughes Medical Institute, Duke Univ. Med. Ctr., Durham, N. C. 27710.

Chronic exposure of cells to β-adrenergic agonists leads to attenuation of the responsiveness of adenylate cyclase (AC) to isoproterenol (iso), termed desensitization. In many cell types, this is accompanied by down-regulation, a decrease in the number of β-adrenergic receptors (βAR) on the cell surface. Recently, our group has shown (Stadel et al. JBC 258: 3021, 1983) that, when frog erythrocyte plasma membranes are prepared by hypotonic lysis of cells followed by vigorous homogenization, the receptors lost from the cell surface can be recovered in a "light membrane fraction" (150,000 x g pellet of an initial 30,000 x g supernatant) where they are sequestered away from the plasma membrane fraction (30,000 x g pellet) and away from the AC and the nucleotide regulatory protein.

We now report that if cells are disrupted by gentler procedures, this "light membrane fraction" containing the sequestered βAR can be demonstrated to be distinct from but physically associated with the plasma membrane. Frog erythrocytes incubated with 10^{-4} M iso showed a 39% reduction in the responsiveness of AC to subsequent stimulation by iso. When βAR number was assessed by the binding of [^{125}I]cyanopindolol to intact cells, the iso-treated cells showed a 41% decrease in the number of cell-surface receptors. When membranes were prepared by gentle lysis (freezing and thawing of the cells with no homogenization), this decrease in receptor number fell to 29% in the plasma membrane fraction (designated P1). When this P1 preparation was vigorously homogenized in the presence of water and centrifuged at 30,000 x g, the majority of the remaining down-regulated βAR were revealed associated with the plasma membrane fraction (designated P2). Thus, this P2 preparation exhibited only a 5% down-regulation of βAR number in iso-treated samples as compared to controls, while still showing a 39% reduction in iso-stimulated AC activity. Furthermore, when cells were desensitized with iso and the βAR from both P1 and P2 solubilized with digitonin, equal numbers of receptors were solubilized from both control and desensitized membranes.

Thus, depending on the precise conditions of membrane preparation, these sequestered βAR can be found either associated with the plasma membrane or in a "light membrane fraction." These data are consistent with a novel mechanism of βAR down-regulation which appears to involve the sequestration of the receptors away from the cell surface in a membrane compartment which remains physically associated with the plasma membrane and which is exposed only upon vigorous shearing of the cell membrane.

325.11 ALPHA-2 ADRENERGIC RECEPTORS: PRESENCE AND FLOW IN SCIATIC NERVE. J. R. Unnerstall, M. A. Zarbin and M. J. Kuhar. Johns Hopkins Univ., School of Medicine, Dept. Neuroscience, Balto., MD 21205.

In this report, the identification and characterization of alpha-2 adrenergic receptors in transport within the ligated rat sciatic nerve will be presented. These receptors were labeled by the alpha-2 antagonist [^{3}H]-rauwolscine ([^{3}H]RW) and detected by light microscopic autoradiographic techniques.

The techniques utilized here have been previously described in detail (Young et al., Science 210:76, 1980; Wamsley et al., Brain Res. 217:155, 1981; Zarbin et al., J. Neurosci. 2:934, 1982). Except where indicated, the binding of [^{3}H]RW (1.0 nM) was carried out in a Na^+-free (K^+-substitute) Krebs-PO_4 buffer at room temp. Sections were preincubated in a normal Krebs-PO_4 buffer with 100 µM GTP for 30 min prior to binding. Sections were washed for 10 min in cold buffer to reduce nonspecific binding which was assessed in the presence of 10 µM phentolamine.

The binding of [^{3}H]RW both proximal (P) and distal (D) to the ligature was time dependent. The binding P to the ligature was disrupted by injections of colchicine into the ligated nerve. Further, binding sites accumulated on both P sides of a double ligature. These data indicate that the [^{3}H]RW binding sites move anterogradely by fast-transport mechanisms. The binding to both sides of the ligature was of high-affinity (K_D's = 1.1 nM) and saturated at 10 nM [^{3}H]RW. The relative potencies of several adrenergic drugs at these binding sites were determined by comparing percent displacements at a constant concentration of unlabeled drug (1.0 µM): agonists, clonidine > alpha-methylnorepinephrine (alpha-MeNE) >> phenylephrine; antagonists, yohimbine $\geq$ spiperone > prazosin > propranolol. Thus, these binding sites appear to have a pharmacology appropriate to the alpha-2 receptor. Displacements using the agonist alpha-MeNE were carried out under various conditions to study the biochemistry of these receptors. Under control conditions, alpha-MeNE was approximately 10 times more potent at P receptors (IC_{50} = 67 nM) than D receptors. In the presence of 30 µM GppNHp, the potency of alpha-MeNE at the P sites was reduced 15-fold, while that at the D sites was reduced only two fold. In the presence of 150 mM Na^+, the potency of alpha-MeNE at both P and D receptors was reduced to equivalent µM levels. The binding of [^{3}H]RW was not significantly altered by either Na^+ or GppNHp.

The data show that alpha-2 receptors are transported within the rat sciatic nerve and represent the first biochemical characterization of the isolated presynaptic alpha-2 receptor. Further, these data show that the biochemical character of the alpha-2 receptor may change during its life cycle, a phenomenon seen with other receptor systems (Zarbin et al., J. Neurosci. 2:934, 1982).

Supported by MH25951, MH00053 and a McKnight Foundation grant.

325.12 EFFECTS OF RESERPINE ON THE AXONAL TRANSPORT AND NUMBER OF β-ADRENORECEPTORS IN RAT BRAIN. E. Agykum*, Anat Biegon and B. E. Levin. (SPON: S. D. Cook). Neurology Serv., VA Med. Ctr., E. Orange, NJ 07019, Dept. of Neurosciences, NJ Med. Sch., Newark, NJ 07103, and Dept. Pharm., Hoffmann-LaRoche, Inc., Nutley, NJ 07110.

A single injection of reserpine (5 mg/kg, i.p.) depleted frontal cortex norepineprine by 10-70% over 6 wk in Sprague-Dawley rats (200-300 g). By 7 d post-reserpine, the B_{max} for {^{3}H} dihydroalprenolol binding to β-adrenoreceptors in the anterior hypothalamus was increased 86% without change in K_d. B_{max} returned to control levels and then increased again by 63% at 21 d. Similarly, the B_{max} for β-receptors in frontal cortex increased by 130% from 4-14 d post-reserpine, returned to control at 21 d and rose again by 67% at 28 d, 7 d later than the second peak seen in anterior hypothalamus. Axonal transport of β-adrenoreceptors was assessed using in vitro binding and autoradiographic localization of {^{3}H} dihydroalprenolol and {^{125}I}-iodopindolol to measure the accumulation of binding sites proximal to a 6-hydroxydopamine lesion in the ascending noradrenergic neurons of the locus coeruleus. Transport of β-adrenoreceptors completely stopped from 7-14 d post-reserpine but then increased to 320% of control at 21 d, the same time at which the second increase of β-adrenergic binding was seen in the anterior hypothalamus and just preceding the second frontal cortex increase. This suggests that the early post-reserpine increases in brain β-adrenoreceptor binding seen at 4-14 d was due to up-regulation of the number of receptors on post-synaptic cells while the second increase seen at 21-28 d was due to receptors synthesized in the locus coeruleus and transported to nerve terminals in the anterior hypothalamus and frontal cortex.

325.13 ACTH ADMINISTRATION ALTERS BRAIN β-ADRENERGIC RECEPTOR AFFINITY AND FUNCTION. R.S. Duman* and S.J. Enna (SPON: S.J. Strada). Dept. Pharmacol. and Neurobiol., Univ. Texas Med. Sch., Houston, TX 77025.

Several studies have suggested that ACTH and corticosteroids influence β-adrenergic receptor function in brain. Thus, adrenalectomy increases, and ACTH treatment decreases the ability of norepinephrine (NE) to stimulate cAMP production in brain slices without altering the number of receptor recognition sites. The present investigation was undertaken to determine the mechanism of this effect. Male Sprague-Dawley rats (125-150 g) received daily s.c. injections of ACTH (Acthar Gel, 50 IU/kg). Control animals received s.c. injections of saline. Sixteen to 18 hr after the last treatment the animals were sacrificed by decapitation and the frontal cortex removed. The ability of NE to inhibit β-adrenergic receptor binding in frontal cortex membrane fragments was analysed using ^{3}H-dihydroalprenolol (^{3}H-DHA) as a ligand. This assay was conducted using various concentrations of NE (1.0 nM to 100 μM) in the presence and absence of Gpp(NH)p. NE-stimulated cAMP production was examined in brain slices using a prelabeling technique. The concentration of NE necessary to inhibit ^{3}H-DHA binding 50% (EC_{50}) was increased 2-fold ($p < 0.05$) following 5 to 7 days of treatment with ACTH. There was also a 2-fold increase in the EC_{50} of NE to stimulate cAMP production, and there was a significant reduction in the maximal response to NE in animals treated with ACTH. Furthermore, while Gpp(NH)p caused a 4-fold increase in the EC_{50} value for NE as an inhibitor of ^{3}H-DHA binding in controls, only a 2-fold shift was detected in brain tissue obtained from animals treated with ACTH. These results suggest that ACTH administration decreases the sensitivity and total responsiveness of the NE-stimulated cAMP system in brain by reducing the receptor affinity for agonists. Since the shift in agonist affinity is not additive with guanine nucleotides it would appear that the hormone treatment influences the interaction between the receptor recognition site and G/F regulatory protein. While it is unclear whether this effect can explain the decrease in the maximal accumulation of cAMP, it could account for the decrease in the sensitivity of the NE receptor-coupled cyclic nucleotide system. These data lend further support to the notion that brain neurotransmitter receptor systems are subject to modification by peptide and steroid hormones. (Supported, in part, by USPHS grants NS-00335, MH-36945 and GM-07405).

325.14 EFFECTS OF LANTHANUM (La^{3+}) ON ALPHA-1-ADRENERGIC STIMULATED PHOSPHATIDYLINOSITOL (Ph.I.) TURNOVER IN RAT HEPATOCYTES. C.A. Harrington* (SPON: P. Kralik) Analytical Neurochemistry, Tex. Res. Inst. of Mental Sci., Houston, TX 77030.

Numerous studies by several laboratories have shown that alpha-adrenergic receptor stimulation is coupled to increased cytosolic Ca^{2+} levels and increased Ph.I. labeling in many cell types. Consideration of this phenomenon in isolated rat hepatocytes and hepatocyte membranes indicates that increases in the level of soluble calcium are probably not causal with respect to the increase in Ph.I. turnover. To further investigate the participation of calcium in alpha-1-adrenergic stimulated phosphatidylinositol turnover we studied the effect of $LaCl_3$ on Ph.I. turnover in isolated rat hepatocytes.

We have found that 1 mM $LaCl_3$ inhibits Ph.I. turnover induced by 100 μM phenylephrine 100%. The ED_{50} for $LaCl_3$ inhibition is 500 μM. Cells washed free of medium La^{3+} and resuspended in a Ca^{2+} containing buffer show no increase in Ph.I. turnover in the presence or absence of maximally stimulating concentrations of phenylephrine. Addition of the divalent ion ionophore A23187 and Ca^{2+} to $LaCl_3$ treated cells also did not stimulate increased Ph.I. labeling with or without phenylephrine.

Cells that we treated with $LaCl_3$ were subsequently fixed with glutaraldehyde, treated with osmium tetroxide, embedded in Epon 812, cut into 200-500 A sections and mounted for electron microscopic analysis. Cells prepared in this fashion exhibited electron dense grains on the plasma membrane. Electron dense material was absent from cells that were untreated with $LaCl_3$. When cells that were damaged were exposed to La^{3+}, deposition of electron dense material was extensive throughout the cytoplasm.

This data does not support the hypothesis that changes in cytosolic Ca^{2+} levels are the required for alpha-1-adrenergic stimulation of Ph.I. turnover in the hepatocyte. Secondly, La^{3+} may be inhibiting Ph.I. turnover by interfering with a Ca^{2+} requiring intramembrane receptor dependent event. The pool of Ca^{2+} involved in this receptor mediated event is not in rapid equilibrium with extracellular or intracellular Ca^{2+}.

325.15 α-ADRENERGIC ACTIVATION AND INDUCTION OF PHASIC AND BURSTING ACTIVITY PATTERNS IN HYPOTHALAMIC SUPRAOPTIC NUCLEUS NEURONS RECORDED IN-VITRO. J.C.R.Randle*, C.W.Bourque* and L.P.Renaud(spon: D.W.Baxter) Neurosciences Unit, Montreal General Hospital and McGill University, Montreal, Quebec, Canada H3G 1A4.

The recent anatomical studies of Sawchenko & Swanson (Science 214:685-687, 1981) have indicated that the dense catecholaminergic innervation of the mammalian supraoptic nucleus (SON) originates predominantly from A1 noradrenergic neurons in the ventrolateral medulla. However, the functional role of noradrenaline (NA) in regulating the excitability of SON neurons remains controversial. For example, in-vivo microiontophoretic application of NA suggests a predominantly inhibitory role (cf Barker et.al.J.Physiol.,218: 19-32,1971) whereas electrical stimulation in A1 selectively activates SON vasopressinergic neurons (Day & Renaud, Soc. Neurosci. Abstr.8:422,1982). We therefore examined the actions of known concentrations of NA and receptor selective agonists applied to SON neurons maintained in-vitro in perfused explants of rat hypothalamus.

Extracellular recordings were obtained from 38 neurons in the ventral and caudal SON, an area of the nucleus containing a predominance of vasopressinergic neurons. NA, methoxamine, or isoproterenol in final concentrations of 10-200 μM were added either in the perfusion media or applied by pressure ejection. Both NA and methoxamine, but not isoproterenol, were observed to enhance the intraburst firing of cells that displayed spontaneous phasic activity (n=9) and prompted both an activation and the appearance of phasic or bursting activity from otherwise quiescent neurons (n= 20). These effects could be blocked by prior application of phenoxybenzamine (2-10μM).

Intracellular recordings were obtained from an additional 12 SON neurons. Both NA and methoxamine, but not isoproterenol, in 10μM concentrations prompted the appearance of bursting in continuously-active cells. At higher concentrations, cells displayed a dose-dependent depolarization of 2-17mV accompanied by a reduction in input resistance up to 30%. In the presence of α-adrenergic agents, action potential durations measured at 1/3 peak amplitude were 10-20% longer, as was the spectrum of spike broadening observed at the onset of phasic bursts.

These observations support a predominantly excitatory role for NA on SON neurons, acting through an α-adrenergic receptor. Moreover, NA appears to induce bursting or phasic activity, patterns consistent with enhanced calcium entry (as a result of a prolongation of the action potentials) and hormone release from neurosecretory terminals in the posterior pituitary.

Supported by the Canadian MRC and F.R.S.Q.

325.16 DIFFERENTIAL SENSITIVITY OF ADRENERGIC MELANOPHORE RECEPTORS IN TWO COLOR MORPHS OF THE CICHLID HAPLOCHROMIS BURTONI. L.E. Muske and R.D. Fernald. Inst. of Neurosci., Univ. of Ore., Eugene, Ore. 97403.

Many teleost fish display bright color patterns and are able to change color quickly. Haplochromis burtoni, a colonial African cichlid, exhibits a variety of colorations associated with age, sex and social status. "Barred" territorial males have a conspicuous dark melanophore stripe (the "eyebar") that can appear and disappear within seconds, and is used as a social signal (Leong, 1969). Some males, called "barless" never display this color pattern, although both melanophores and functional nerves controlling them are present in the eyebar region (Muske and Fernald, 1981). Except for the eyebar, barred and barless males appear identical, and there are no intermediate morphs.

Rapid darkening or lightening in teleosts is controlled by noradrenergic sympathetic fibers innervating dermal melanophores. Electrical stimulation of chromatic motor fibers causes pigment aggregation (paling). The hormone epinephrine has similar effects in many species, and has also been implicated in the opposite (rapid darkening) response (Miyashita and Fujii, 1975). This study used in vitro preparations of eyebar tissue to examine whether there are differences in the properties of adrenergic receptors mediating melanosome aggregation in the eyebar that might account for the morphological difference between barred and barless males.

Eyebar melanosomes disperse in physiological saline. Both epinephrine and norepinephrine elicit noticeable pigment aggregation at concentrations as low as 10^{-9} M. Barless males are significantly more sensitive to low (5×10^{-8} - 10^{-7} M) concentrations of the neurotransmitter than barred, and this effect appears to be unique to melanophores present in the eyebar. In both males, melanosome aggregation is mediated by alpha adrenergic receptors. Preliminary evidence also indicates that whereas in barred males, norepinephrine is more effective in initiating this response, the relative effectiveness of these two agonists may be reversed in barless, suggesting the possibility that there may likewise be differences between the two morphs in B adrenergic activity.

This evidence, of an intraspecific difference in the properties of receptors that is correlated with an important color dimorphism, has not been described previously. These results suggest a plausible mechanism to explain why, in the same social and motivational context, barred and barless males exhibit this difference in coloration.

Supported by NIH 5T32 GM07257.

325.17 **LONG-TERM UNILATERAL NORADRENERGIC DEAFFERENTATION OF THE RAT NEOCORTEX: MONOAMINE CONTENT AND ^{3}H-PRAZOSIN BINDING SITES.** T.A. Reader and R. Brière*. Centre de recherche en sciences neurologiques, Département de physiologie, Université de Montréal, Montréal, Québec, Canada.

Direct biochemical determinations of α_1-adrenoceptor sites were performed in the neocortex of rats subjected to a selective unilateral noradrenergic deafferentation, obtained by microinjecting 6-hydroxydopamine (6-OHDA; 2µg/2µl) in the right dorsal noradrenergic bundle (DNB), at the level of the superior cerebellar peduncle (A=0.16; L=1.75 and H=-1.60 mm [1,4]). After 3 months survival the α_1 sites were assayed [3] using ^{3}H-Prazosin (^{3}H-PRZ), in both the deafferented and the contralateral (control) cortex. In order to assess the extent and specificity of the deafferentation, the catecholamines and serotonin (5-HT) were measured using radioenzymatic assays [2] in samples from the frontal cortex, septum and hippocampus.

Monoamine levels and ^{3}H-PRZ binding sites (n=8)

	NE ng/mg P	DA ng/mg P	5-HT ng/mg P	B_{max} fmol/mg P
Control	2.57 ± 0.17	0.54 ± 0.10	3.17 ± 0.45	164 ± 21
Lesioned	0.80 ± 0.30	0.74 ± 0.12	3.00 ± 0.36	238 ± 34
% change	-68.74**	+38.06*	NS	+44.76*

Statistical significance was calculated by paired-t-statistical analysis *$p < 0.05$; and **$p < 0.001$.

In the samples of septum and hippocampus from the lesioned hemispheres only significant reductions (-58.16 and -70.21%, respectively) in NE content could be detected, when compared to the paired samples obtained from the control side.

The results document that unilateral NE-deafferentations of the cerebral cortex are feasable, the reduction in NE persists for at least three months, and there is an increased DA content. In the denervated neocortex specific ^{3}H-PRZ binding showed an increase in B_{max}, without any changes in the dissociation constants (K_d 25°C of 0.217 ± 0.082 and 0.241 ± 0.045 nM, for lesioned and control samples respectively). The results have to be considered in relation to the plasticity of monoaminergic fibers [2,4] and to denervation-induced alterations of postsynaptic α-adrenoceptors.

References: 1) König JFR, Klippel RA. The Rat Brain: A Stereotaxic Atlas, NY, Krieger, 1963. 2) Reader TA, Brain Res Bull 8, 527-534. 1982. 3) Reader TA, Brière R, Brain Res Bull 10, 155-158, 1983. 4) Tassin et al, Neuroscience 4, 1569-1582, 1979.

(Supported by the MRC [MA-6967]).

325.18 EFFECTS OF REPEATED ELECTROCONVULSIVE SHOCKS ON NEUROTRANSMITTER TURNOVER IN RAT BRAIN. E.R. Korpi*, F. Karoum, L.-W. Chuang*, M. Linnoila* and R.J. Wyatt. Adult Psychiatry Branch, NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

Repeated electroconvulsive shocks (ECS) have been shown to reduce the number of β -adrenergic receptors and to increase the number of serotonin S-2 receptors in certain brain areas. While the receptor changes apparently can explain behavioral responses to noradrenergic and serotonergic agonists, the mechanism(s) of how these changes are produced is unclear. Theoretically, decreased and increased densities of postsynaptic β - and S-2 receptors, respectively, may be associated with reciprocal changes in the turnover rates of the amines.

To test the above hypothesis, we gave seven ECS to adult male rats during a period of ten days. The rats were lightly anaesthetized with ether, and the ECS were delivered through ear clips for a period of 1 s using a Medcraft M-24 ECT Unit set at 150 V. The animals were sacrificed 24 h after the last ECS, and hypothalamus, corpus striatum, frontal cortex and hippocampus dissected out for biochemical analysis. Catecholamines and their metabolites were measured by mass-fragmentography, and serotonin by reversed phase liquid-chromatography with electrochemical detection. The turnover of the catecholamines was estimated by following the decline in norepinephrine and dopamine metabolites concentrations after intraperitoneal injection of pargyline (75 mg/kg body weight), and by equating the changes in the formation rates of the metabolites to similar changes in the turnover rates of the parent amines. The increment of serotonin concentration after pargyline injection was taken as a measure of serotonin turnover.

Steady state concentration of serotonin in the frontal cortex was slightly increased in the ECS rats. No differences were detectable in the concentrations of dopamine and norepinephrine in hypothalamus, corpus striatum, hippocampus or frontal cortex. Norepinephrine turnover was enhanced in hypothalamus and frontal cortex, and serotonin turnover was decreased in frontal cortex of treated animals as compared with sham-treated controls. The results will be discussed of how they support the idea that the changes in noradrenergic and serotonergic receptors after electroconvulsive treatments may be secondary to alterations in the turnover rates of these amines in specific brain regions.

325.19 EFFECTS OF A HIGH NaCl DIET ON KIDNEY AND BRAIN ADRENERGIC RECEPTOR BINDING: Na TREATMENT IN VIVO ALTERS THE EFFECTS OF Na AND GTP IN VITRO. P. Ernsberger and D.C. U'Prichard, Dept. of Pharmacology, Northwestern Univ., Chicago, IL 60611.

Sodium ion decreases agonist affinities in several receptor systems in vitro. We examined the effects of Na treatment in vivo on kidney and brain adrenergic receptor density and ion and nucleotide sensitivity. Male rats were fed either 0.6 or 4.0% NaCl chow for 1 or 3 weeks prior to sacrifice. Tail blood pressure was monitored weekly. We examined α_1-receptors using ^{3}H-prazosin (^{3}H-PRAZ), high-affinity α_2 sites using ^{3}H-p-aminoclonidine (^{3}H-PAC), low-affinity α_2-sites using ^{3}H-rauwolscine (^{3}H-RAUW), and β-receptors using ^{125}I-iodocyanopindolol. Kidney α_1-receptor density was increased by 1/4 to 1/3 after 1 or 3 weeks of the high Na diet. The increase was greatest in those rats displaying the greatest blood pressure increment following the introduction of the diet. ^{3}H-PAC binding increased nearly 50% after Na treatment for 1 or 3 weeks, and ^{3}H-RAUW binding was increased 26% at 1 week. β-receptor density was decreased by 29% at 3 weeks. This decrease was greatest in those rats displaying the smallest blood pressure increment following the introduction of the diet. 25mM Na decreased ^{3}H-PAC binding, and this decrease was 25% greater in rats fed a high Na diet. The inhibition of ^{3}H-PAC binding by 0.1mM GTP was also enhanced after 1, but not 3, weeks of the diet. 0.1mM GTP decreased ^{3}H-RAUW binding, and this effect was enhanced 70% after 3 weeks of Na treatment. The ability of 1µM (-)-norepinephrine (NE) to inhibit ^{3}H-RAUW binding was increased in rats fed a high Na diet (controls: 62±7% inhibition, 1 week: 80±4%, 3 weeks: 81±3%). 100mM Na decreased the ability of 1µM NE to inhibit ^{3}H-PRAZ binding; this effect of Na was enhanced 65% after Na treatment in vivo. 5mM Mg increased the ability of NE to displace ^{3}H-PRAZ binding to a similar extent in all groups. In the cerebral cortex, the high affinity component of ^{3}H-PAC binding was selectively increased after 3 weeks of the high Na diet. Na inhibition of ^{3}H-PAC binding was unaffected by the diet, while GTP inhibition of binding was increased (74% inhibition vs. 52% in controls). The simultaneous inclusion of Mg and GTP increased ^{3}H-PAC binding 3.7-fold relative to GTP alone, while Mg produced a 2.5-fold increase in controls. The present study demonstrates that treatment with a high Na diet enhances the ability of Na in vitro to inhibit both kidney α_2-agonist binding and agonist interactions at α_1 sites labeled by an antagonist radioligand. GTP inhibition of α_2-agonist binding was enhanced by in vivo Na treatment in both the cerebral cortex and kidney. The results are consistent with an enhancement of high-affinity agonist interactions at α_1- and α_2-receptors following a high NaCl diet.

325.20 BETA-ADRENERGIC RECEPTORS IN CORTEX AND HYPOTHALAMUS OF HEMIDECORTICATE RATS. J.C. Bedran de Castro*, S.L. Petrovic* and S.M. McCann. Department of Physiology, University of Texas Health Science Center at Dallas, Dallas, TX 75235.

Suppression of cortical and rhinencephalic function by hemidecortication results in alterations in weight of several endocrine glands and of accessory sex organs, of the estrous cycle and also leads to an increased synthesis and release of gonadotropins. Many experiments have been performed to show the anatomic correlations and physiological role of catecholaminergic mechanisms in gonadotropin release. The purpose of the present study was to determine the concentration of β receptors in cortex and hypothalamus of long term (at least 30 days) hemidecorticated animals which exhibited elevated plasma gonadotropin levels as observed previously. The density of β receptors was measured using a high-affinity β adrenergic ligand, iodocyanopindolol (ICYP). The results show an increase of 36% of cerebral cortical β-receptors in hemidecorticated rats above controls in proestrus and about a 20% increase after ovariectomy in the remaining cortex (right cortex). In the ipsilateral (left) half of the hypothalamus of hemidecorticated, ovariectomized rats, the number of β receptors was 41% less than that in the right half of the hypothalamus. When compared with sham-operated rats, the β receptor density was 18% lower in the left half, whereas it was 15% above the controls in the right half. All the differences were statistically significant ($p<0.01$). We postulate that increases in β receptor numbers indicate a response to reduction of noradrenergic input to a region, whereas decreases represent a response to increased noradrenergic drive. Thus, it follows that there is a direct or indirect adrenergic pathway from left to right cerebral cortex which is interrupted by hemidecortication, resulting in an increase of beta-receptors in the remaining cortex. There must also exist a crossed pathway to the contralateral hypothalamus to account for the increase in beta-receptors in that part of the hypothalamus following hemidecortication. The decrease in hypothalamic beta-receptors on the ipsilateral side suggests an increased noradrenergic input to this region. (Supported by FAPESP and HD-09988).

326.1 SIMULTANEOUS ACTIONS OF DEXAMETHASONE ON NOREPINEPHRINE NEUROCHEMISTRY, ACTH RELEASE, AND NORADRENERGIC SUBSENSITIVITY IN THE RAT. E.A. Muth, J.A. Moyer, J.T. Haskins, and E.B. Sigg*. Dept. of Experimental Therapeutics, Wyeth Labs., Philadelphia, PA 19101.

Many depressed patients show a rapid escape, compared to normal individuals, from the suppression by dexamethasone (DEX) of corticosteroid (CS) release. Most antidepressants (AD) suppress histamine-induced ACTH/CS release in rodents (Reilly & Sigg, JPET 222:583-588, 1982). These same AD's cause chronic desensitization of the noradrenergic (NE) β-receptor-linked cyclic AMP (cAMP) generating system in brain, presumably resulting from their potentiation of NE and/or serotonin (5-HT) synaptic transmission. We have continued preliminary experiments designed to uncover the link between AD effects on NE/5-HT transmission, NE subsensitivity, and ACTH release.

It has been shown that DEX, while inhibiting the α-methyltyrosine (αMT)-induced release of CS, concurrently blocks the αMT depletion of hypothalamic NE (Sigg & Keim, Fed. Proc. 37:524, 1978). In the present experiments, the complete blockade by DEX (400 µg/kg s.c.) of the increase in ACTH by αMT (250 mg/kg i.p.) was accompanied by a partial antagonism (34%, $p<0.05$) of the inhibition by αMT of hypothalamic tyrosine hydroxylase (TH) activity. However, DEX up to 100 µM showed no reversal of in vitro TH inhibition by αMT at its IC50 (0.06 mM) in either hypothalamic or striatal homogenates. Furthermore, we were unable to show any inhibition by DEX of NE (also Sigg & Keim, ibid.), 5-HT, or tyrosine synaptosomal uptake mechanisms, which discounts an action by DEX to prevent entry of αMT into the neuron (although perikaryal uptake mechanisms have yet to be examined). On the other hand, DEX applied microiontophoretically inhibited firing in 11 of 22 NE locus coeruleus neurons tested (none were excited). These findings suggest that DEX inhibits NE neuronal firing, resulting in a lack of NE depletion following αMT synthesis inhibition, and that hypothalamic NE has a stimulatory role in ACTH release.

We have further shown that DEX causes pineal noradrenergic downregulation (53 and 41% decrease in isoproterenol-stimulated cAMP) after both acute (400 µg/kg s.c.) and chronic (400 µg/kg s.c./day, 5 days) treatment. Surprisingly, this downregulation was unaccompanied by any significant decrease in pineal β-receptor density. If this downregulation also occurs in the hypothalamus, it may be that DEX acts on NE systems both directly (inhibition of firing) and postsynaptically (cAMP downregulation) to block NE-stimulated ACTH release. Such an action could explain the lack of DEX suppression of CS release seen in depressives who subsequently respond to treatment with AD's which downregulate noradrenergic systems.

326.2 CHLORDIAZEPOXIDE ATTENUATES CRF EFFECTS ON CONFLICT TEST. K. Thatcher Britton*, J. Rivier*, W. Vale, and G.F. Koob. Univ. California, San Diego and VA Hospital, La Jolla, CA 92093, Peptide Biol. Lab and A.V. Davis Centr. for Neurobiol., The Salk Institute, San Diego, CA 92138. (SPON: L. Judd)

Corticotropin-releasing factor (CRF), characterized from ovine hypothalamic extracts, has been proposed to play a role in coordinating endocrine, physiological and behavioral responses to stressful stimuli. It was of interest, therefore, to examine the effect of CRF on a behavioral test in which "anxiety" or conflict influences performance. The effect of chlordiazepoxide on CRF-induced behavioral changes was also examined.

Rats, implanted with intraventricular cannulae, were trained on a Geller-Seifter conflict test modified for incremental shock (Pollard and Howard, Psychopharm. 62, 117-121 (1979)) and consisting of three components: reward, time-out and conflict. Responses during the reward component were reinforced on a random interval schedule--30 sec.; responses during the time out component were never reinforced and responses during the conflict component were continuously reinforced with food and foot shock (scrambled constant current, biphasic, square wave produced by a SGS-003 stimulator-BRS/LVE). The current increased by 0.1 mA with each successive shock during the conflict component until reaching 2.2 mA where it remained for the duration of the conflict period. A session consisted of two cycles each consisting of a 5 min reward period, 2 min time out and 2 min conflict period presented in succession. Following baseline stabilization, rats were injected with the compounds below.

CRF (0.01, 0.1, 1.0 µg icv) significantly decreased both punished and nonpunished responding on the conflict test. This effect was maximal at the highest dose, decreasing nonpunished responding to 60% of baseline and the number of shocks taken to 55% of baseline. Chlordiazepoxide (5mg/kg i.p.) significantly attenuated the response-decreasing effects of CRF (1.0 µg) on both punished and nonpunished responding. These results support the hypothesis that CRF has a role in mediating an organism's behavioral response to stress and "anxiety".

326.3 CORTICOSTERONE LEVELS IN THE LEARNED HELPLESSNESS STRESS PARADIGM. S. Ryan, M. Laudenslager, and S. Maier*. Psychology Dept., Univ. of Colorado, Boulder, CO 80309.

The learned helplessness paradigm produces analgesia and immune responses which depend upon controllability of the stressor. Corticosterone levels were examined to determine their correspondence with these effects. The shock paradigm was identical to that used for analgesia and immune response assessment. On day 1, rats recieved 80 tail shocks in a wheel turn chamber. Shock was either escapable(E) or inescapable(Y); others were restrained but not shocked(R). On day 2, five short "reinstatement shocks"(RS) were delivered through the floor of a shuttlebox. E, Y, and R animals were decapitated at several time points: immediately following treatment, 24 hours later, or immediately following RS on day 2. Corticosterone (C) levels were then measured by radioimmunoassay of plasma samples.

On day 1, both E and Y groups were elevated above R and home cage controls, though all were back to baseline levels 24 hours later. On day 2, E-RS and Y-RS groups were at similar, intermediate levels while R-RS levels were significantly higher than both E-RS and Y-RS groups. These results are interesting with regard to both analgesia and T cell immune responses. In previous analgesia studies, the Y-RS group exhibits opioid analgesia while the E-RS and R-RS groups do not. Adrenalectomy, hypophysectomy, and dexamethasone all block reinstated analgesia from inescapable shock; C therapy in adrenalectomized animals returns it (MacLennan et. al., 1982). However, higher C levels in the R-RS group and lower, similar levels in the E-RS and Y-RS groups suggests that C may be necessary but not sufficient for reinstated analgesia. T-lymphocyte response to mitogen stimulation is suppressed in Y-RS animals, but is normal in R-RS and E-RS rats (Laudenslager, et. al., in press). Though C is often implicated in immunosuppression, relative levels do not support that conclusion here: C levels are significantly elevated in the R-RS group, for which the immune response is normal; C levels are similar for groups E-RS and Y-RS for which immune responses differed. These data suggest that the corticosterone stress response does not seem to play a critical causitive role in either long-term opioid analgesia or T lymphocyte immunosuppression.

326.4 INFLUENCE OF EXPERIMENTAL STRESSORS, LIFE EVENTS AND PERSONALITY VARIABLES TO PLASMA STEROID ALTERATIONS. A. Corradini*, Z. Merali* and H. Anisman. Dept. of Psychology, Carleton University, Ottawa, Ontario K1S 5B6, Canada.

Exposure to aversive events are thought to be fundamental to the provocation or exacerbation of some psychological and physical pathologies owing to the neurochemical and hormonal/steroid alterations provoked by the insults. The effectiveness of stressors in provoking pathology appear to be determined, in part, by numerous environmental, experiential and organismic variables. In order to assess the relationship between life-stress, personality variables (e.g., Type A and B patterns, trait and state anxiety, locus of control), reactivity to stressors, and affective state, a prospective study using university students was conducted over a 5 month period. Subjects were required to report their previous life stress history and, in addition, monitored their "Daily Hassles" and "Uplifts". Subjects were subsequently tested in a maze completion task, where mazes were either solvable or insolvable. Prior to and during the problem-solving tasks blood samples were collected at five intervals, for determination of corticoids, prolactin, epinephrine and norepinephrine. The problem-solving task was found to influence serum corticoid concentrations. Moreover, the extent of the change varied as a function of whether the task was solvable or insolvable. Prolactin concentrations, in contrast, were not influenced by either the solvable or insolvable problem. Variations of corticoid concentrations were related to the individuals' stress history. The steroid reactivity may have been a reflection of the subjects' perception or interpretation of environmental events, as opposed to the stressor per se. The data were discussed in terms of individual differences in the perception of aversive events, the contribution of stress controllability and mastery to steroid/hormone reactivity, and the relationship of these variables to the induction or exacerbation of illness.

326.5 ENDOCRINE ACTIVATION, BUT NOT TAIL-FLICK LATENCY ALTERATION, INDUCED BY CONSUMPTION OF CONCENTRATED SUCROSE SOLUTION.
G. D. Coover, G. T. Satterfield*, W. P. Smotherman, D. Steinke* and D. M. Dorsa*. Depts. of Psychology, Northern Illinois Univ., DeKalb, IL 60115 and Oregon State Univ., Corvallis, OR 97331 and VA Medical Center, Seattle, WA 98108.

Consumption of sucrose solution can elevate the plasma concentration of corticosterone in rats. The elevation is enhanced by prior water deprivation and greater sucrose concentration in the range of 6% to 24% w/w sucrose, and can equal in magnitude corticosterone responses to severe stressors (Hart, Coover, Shnerson and Smotherman, J. comp. physiol. Psychol. 94: 337-345, 1980). Three experiments examined whether sucrose consumption elevates tail-flick latencies and plasma beta-endorphin levels, which are commonly reported sequelae of stress.

General procedures included pre-exposure of the rats to the solution and pretest (baseline) tail-flick measurement (IITC, Inc. Model 33 analgesia meter). The rats were deprived of water for 24 or 36 hr prior to solution presentation. Blood samples were obtained by decapitation immediately following solution consumption or tail-flick testing post-consumption.

In Exp. 1, rats were provided 0%, 4% or 24% sucrose for 50 min. Three tail-flick trials were spaced over 10 min such that the rats were decapitated at Time 60 min. Plasma corticosterone concentrations (µg/100 ml) were higher in rats that consumed the 24% solution (Mean = 51.6, SE = ±5.8) than in those that consumed the 4% (29.9 ±1.0) or 0% (28.4 ±2.8) solution. Tail-flick latencies (sec) were not affected by the type of solution consumed (3.78 ±.17, 3.89 ±.18 and 3.82 ±.16, respectively).

In Exps. 2 and 3, only the 24% solution was used. The rats were water deprived for 36 hr before presentation of the sucrose solution for 0, 20, 60 or 180 min. In Exp. 2 three tail-flick measurements were made within 2 min of test solution removal prior to decapitation, while in Exp. 3 rats were decapitated immediately following test solution removal. Corticosterone levels of the tail-flick tested rats of Exp. 2 for the 0-, 20-, 60- and 180-min groups were 9.8 ±1.4, 34.5 ±2.8, 42.4 ±6.9 and 6.0 ±1.4, respectively. Plasma glucose concentrations (mg/100 ml) of these rats were 129 ±4, 175 ±18, 140 ±7 and 152 ±8. The tail-flick latencies did not differ: 3.81 ±.52, 4.08 ±.40, 4.31 ±.19 and 4.27 ±.19, respectively. The plasma beta-endorphin levels of the rats in the comparable four groups of Exp. 3 are presently being assayed.

The present results do not support a comprehensive analgesia mediated by hypothalamo-pituitary beta-endorphin activation. However, important questions remain as to the mechanisms and effects of the endocrine activation produced by consumption of concentrated sucrose solution.

326.6 ADRENALECTOMY ABOLISHES STRESS-INDUCED INCREASE IN PITUITARY CYCLIC AMP. J.L. Meyerhoff, G.J. Kant, C.J. Nielsen*, E.H. Mougey* and L.L. Pennington*. Neuroendocrinology and Neurochemistry Branch, Dept. Medical Neurosciences, Div.Neuropsychiatry, Walter Reed Army Institute of Research, Washington, DC, 20307.

We have previously demonstrated that various stressors increase pituitary cyclic AMP in vivo in the rat (Kant, Meyerhoff, Bunnell and Lenox, Pharmacol. Biochem. Behav., 17, 1067-1072, 1982). To understand the mechanisms mediating this response, we have examined the effect of adrenalectomy on footshock (FS) induced increases in pituitary cyclic AMP. Male Sprague Dawley rats were subjected to adrenalectomy under pentobarbital anesthesia. These rats plus a control group were maintained on a drinking solution of 0.9% sodium chloride for the remainder of the experiment (1 wk), while a third group of controls were maintained on tap water. All rats were handled daily during this week, and were habituated to entering a lucite tube which resembled the tube used to immobilize them for sacrifice by microwave irradiation. On the day of the experiment, each of these three groups was divided into two subgroups: FS vs No Shock. The FS rats were subjected to unavoidable, variable interval footshock at 1.6 mA intensity for 15 minutes, then sacrificed immediately. The unshocked rats were sacrificed immediately upon removal from the home cage. Following sacrifice by exposure to 5 sec of high-power focussed microwave irradiation, the rats were decapitated, trunk blood was collected for hormone assays and the pituitaries were dissected, weighed and sonicated in sodium acetate buffer. The supernatants were assayed for cyclic AMP by radioimmunoassay (Lenox, et al., Neuroendocrinology 30: 300-308, 1980).

Pituitary cyclic AMP (picomole/mg wet wt.)

	N	WATER CONTROL	SALINE CONTROL	SALINE ADREX
NO SHOCK	6	1.31 ± 0.14	1.06 ± 0.08	1.31 ± 0.06
FOOTSHOCK	6	6.16 ± 1.50	12.65 ± 2.34	1.19 ± 0.15

Adrenalectomy completely abolished the stress-induced increase in pituitary cyclic AMP, along with the plasma corticosterone response. The stress induced elevation in plasma prolactin was not affected. Drinking saline instead of tap water appears to exaggerate the stress-induced increase in pituitary cyclic AMP. Further studies have shown that adrenal medullectomy also abolishes the pituitary cyclic AMP response to stress. Our results indicate that adrenal factors mediate the stress-induced increase in pituitary cyclic AMP, but do not establish whether they do so independently of hypothalamic influences.

326.7 DEXAMETHASONE DOES NOT SUPPRESS STRESS INDUCED INCREASES IN PITUITARY CYCLIC AMP. E.H. Mougey*, D.R. Collins*, L.L. Pennington*, W.L. Gamble*, G.J. Kant, J.L. Meyerhoff. (SPON: H.C. Holloway) Dept. of Medical Neurosciences, Div. of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D.C. 20307.

We have shown that footshock elevates levels of pituitary cyclic AMP in vivo (Kant et al. Pharmacol. Biochem. Behav., 17:1067,1982). Since footshock also causes the release of β-endorphin (β-EP) and ACTH from the pituitary (Rossier et al. Nature, 270:618, 1977), and since CRF (corticotropin releasing factor), in addition to causing release of pituitary hormones in vivo, elevates levels of pituitary cyclic AMP in vitro (Labrie et al. Science, 216:1007, 1982), it seemed desirable to determine if the stress-induced increase of pituitary cyclic AMP and the release of these pituitary hormones were interrelated. Dexamethasone has been shown to suppress stress-induced release of β-EP and ACTH from the pituitary. We conducted the following experiments to determine if dexamethasone would also suppress the pituitary cyclic AMP response to stress.

Male Sprague-Dawley rats (300 ± 20g) were housed in individual cages and handled daily during the four days prior to sacrifice to minimize non-specific stress effects. Twenty-four hours before the experiment, half of the rats received an IP injection of dexamethasone (0.4 mg/Kg) and two hours prior to sacrifice they received an additional dose of 0.2 mg/Kg. The remaining group of rats (controls) received saline injections on both occasions. Half of the dexamethasone pretreated rats and half of the control rats were then subjected to footshock for 15 min. on a variable interval schedule. Immediately following footshock, the animals were sacrificed by either microwave irradiation or decapitation. The non-shocked rats were sacrificed in the same manner immediately after removal from their home cages. The pituitaries were removed from the microwaved animals, weighed, sonicated in acetate buffer and the supernatants were assayed for cyclic AMP by radioimmunoassay. Plasmas obtained from these animals were assayed for corticosterone and prolactin by radioimmunoassay. Plasmas collected from the decapitated animals were assayed for corticosterone, prolactin, β-EP and β-LPH by radioimmunoassay.

Footshock increased the levels of all indices measured. Partial suppression of the β-EP (48%) and corticosterone (42%) stress responses was observed in the dexamethasone pretreated animals and significant supression of resting levels of β-EP (35%) and corticosterone (72%) in unshocked rats was noted. Stress-induced prolactin increases, however, were not attenuated by this dose of dexamethasone contrary to data reported by Rossier et al. (Proc. Natl. Acad. Sci., 77:666, 1980). Similarly, the pituitary cyclic AMP increases found in footshocked animals were unaffected by dexamethasone pretreatment. In view of the above findings it would appear that the concentration of cyclic AMP found in the pituitary following stress is unrelated to circulating levels of adrenocortical hormones.

326.8 THE EFFECTS OF FOOTSHOCK STRESS ON GLUCOCORTICOID RECEPTORS IN RAT HIPPOCAMPAL CYTOSOL. C.J. Nielsen* and G.J. Kant. (SPON: G.R. Sessions). Neuroendocrinology and Neurochemistry Br., Dept. Med. Neurosciences, Div. Neuropsychiatry, Walter Reed Army Institute of Research, Washington DC 20307.

Footshock increases the release of hormones from the pituitary and adrenal glands of the rat, including ACTH from the pituitary and corticosterone from the adrenal gland. Corticosterone feeds back and inhibits the release of ACTH, thereby decreasing the further release of corticosterone.

Glucocorticoids have been shown to bind preferentially to receptors in the hippocampus [McEwen et al., Brain Res. 17 471 (1970)], and it has been suggested that the hippocampus is an important link in the feedback loop between the pituitary and adrenals McEwen, Ann NY Acad. Sci. 297,568 (1977). We have begun a series of experiments designed to characterize the effects of stress-induced release of corticosterone on hippocampal glucocorticoid receptors.

Male Sprague-Dawley (250-350g) rats were used in these studies. In some experiments, rats were adrenalectomized 1 week prior to sacrifice and maintained on saline. All rats were handled for 4 days prior to each experiment to minimize non-specific stress effects. Control rats were sacrificed by decapitation immediately upon removal from their home cage. Other rats were subjected to footshock on a variable interval schedule with an average intershock interval of 30 sec. Shock duration was 5 sec . Except as otherwise stated, the shock session length was 15 min and the shock intensity was 1.6 mA. Shocked rats were sacrificed immediately after the end of the shock session. After decapitation, the hippocampi were dissected on ice, weighed, and homogenized at 0° in 2 volumes of 10 mM Hepes-molybdate buffer according to the method of Housley et al. (J.Biol. Chem. 257,8615 (1982). The specific binding capacity of the supernatant prepared from this homogenate was determined by incubating aliquots with 50 nM ^{3}H-triamcinolone acetonide in the presence or absence of a 1000-fold excess of unlabelled dexamethasone. Unbound steroid was removed by incubation with 1% dextran-charcoal.

Adrenalectomy doubled the binding capacity of the hippocampal cytosol as compared to normal animals. Footshock markedly decreased the binding capacity of normal rats (by 50%) , but did not affect glucocorticoid binding in adrenalectomized rats. The shock- induced decrease in hippocampal glucocorticoid binding capacity appeared to be dependent upon shock intensity and session length .

These data suggest that the stress-induced release of corticosterone from the adrenal glands triggers the subsequent rapid translocation of glucocorticoid receptors from the cytosol to the nucleus. This phenomenon is reflected in the loss of measurable cytosol glucocorticoid binding capacity.

326.9 GRADED FOOTSHOCK STRESS ELEVATES PITUITARY CYCLIC AMP AND PLASMA β-ENDORPHIN, β-LPH, CORTICOSTERONE, AND PROLACTIN. G.J.Kant, E.H. Mougey*, D.R. Collins*, C.B.Wormley*, L.L. Pennington* and J.L. Meyerhoff. Neuroendocrinology and Neurochemistry Br, Dept. Med. Neurosciences, Div Neuropsychiatry, Walter Reed Army Institute of Research, Washington DC, 20307.

We previously reported that a variety of stressors including restraint, forced running, s.c. formalin injection, conditioned psychological stress, cold exposure and footshock elevated levels of pituitary cyclic AMP as well as plasma corticosterone and plasma prolactin [Kant et al.,Neurosci Abs. 7,333 (1981); Kant et al., Pharmacol Biochem Behav 17,1067-1072 (1982) ; Bunnell et al. Neurosci Abs. 7, 869 (1982)]. Although the amplitude of the pituitary cyclic AMP response appeared to be highly correlated with the plasma prolactin response and both these measures appeared to correlate with the "severity" of the stressor, the comparison of stressors was clearly subjective.

Therefore, in order to further characterize the role of pituitary cyclic AMP in the hormonal response to stress, we have conducted two complementary studies using one stressor whose severity could be regularly manipulated, footshock. Male rats were subjected to 15 min of various current intensities of footshock (0, 0.2, 0.4, 0.8, 1.6, 2.4, 3.2 mA) on a variable interval schedule with an average intershock interval of 30 seconds. Shock duration was 5 seconds. Animals were sacrificed immediately after being removed from the shock box. In the first experiment , animals were sacrificed by high power microwave irradiation to prevent post-mortem changes in cyclic AMP levels. The pituitaries were dissected, weighed and sonicated in sodium acetate buffer and the supernatants were assayed for cyclic AMP by radioimmunoassay. In the second experiment, animals were sacrificed by decapitation and trunk blood was collected. Plasma hormones were measured by radioimmunoassay.

Increasing intensities of footshock elevated levels of all measured stress indices. The maximal elevations observed were 17 fold for pituitary cyclic AMP, 5 fold for β-Endorphin, 25 fold for β-LPH , 14 fold for prolactin, and 5 fold for corticosterone. Generally, there was little or no pituitary cyclic AMP or neuroendocrine response observed at the lowest current intensities, a proportional response over the medium intensities and a maximal plateau response at the highest intensities. However, there were some differences observed among the different biochemical stress markers with respect to threshold intensity for response, range of proportional response and maximal response. Corticosterone appeared to be a more sensitive responder to mild current intensities than the other indices measured but was less sensitive to differences between higher levels of current. There was a positive correlation among all biochemical indices measured as well as with an observer rated behavioral index of stress response.

326.10 Effect of Diazepam on Heart Rate (HR) in Resting and Stressed Male Rats. S.T. Conahan* and W.H. Vogel, Dept. of Pharmacology, Thomas Jefferson Univ., Philadelphia, PA 19107

Male Sprague Dawley rats weighing about 300 g and carrying indwelling subcutaneous ECG leads (lead 2) were used to determine the effect of diazepam on HR at rest and at 1, 5, 15 and 30 min of immobilization stress. 24 hours following immobilization, the animals received either diazepam (5 mg/kg, I.P.) or vehicle, and the experiment was repeated.

Immobilization produced pronounced tachycardia in all animals in both trials. Second trial resting HR did not differ from first trial resting values (318.7±14.9 vs. 338.7±9.1 (BPM)). Administration of diazepam to the resting animals caused a significant increase in HR (84±18.3 BPM) ($p< 0.05$), while vehicle administration had no effect (24±18 BPM). In animals receiving diazepam, immobilization again produced tachycardia, however, it was significantly less than that observed during the first trial at 1 and 5 min of immobilization ($p< 0.05$). Vehicle alone had no effect on the maximal increase in HR during immobilization.

The resting ECG in the conscious rats resembled the ECG of anesthetized animals previously reported in the literature. During stress, certain ECG abnormalities were observed. Preliminary results indicate that diazepam may effect the ECG in the resting and stressed state.

These data indicate that diazepam's effect on HR may differ depending on the emotional state of the animal; increasing it in the non-stressed state and protecting against the maximal increase in the acute stress situation.

326.11 STRESS RELATED CHANGES IN CORTICAL BENZODIAZEPINE AND HIPPOCAMPAL QNB BINDING. S.I. Dworkin, G.F. Guerin and J.E. SMITH. Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center in Shreveport, LA 71130.

A better understanding of the neurobiological consequences of exposure to noxious or aversive environments would be beneficial to the treatment of "stress-related" pathologies. Therefore, an analysis of central neurotransmitter receptor changes induced by stress-related environments would add to knowledge of stress pathologies. This study examined the effects of noxious stimulation on CNS receptor binding.

Twelve groups of rats containing four littermates each were exposed to four different parameters of a 3-minute random-time schedule of shock presentation: (1) signaled shock (SHCS), (2) unsignaled shock (SH), (3) signal only (CS), and (4) home-cage control (HC). Half of the litters were exposed to one four-hour session, the other half were exposed to four, four-hour sessions. Four additional groups of littermates were exposed to a 30-minute sessions of either a random-time 2.7-second schedule of shock presentation or placed in the experimental chamber (high density or HD and high density control or HDC, respectively). Immediately after each experimental treatment, the rats were sacrificed by decapitation and the cerebral cortex and hippocampus were isolated by dissection. Trunk blood from the cervical wound was collected in heparinized tubes and centrifuged.

Plasma corticosterone levels were determined using a cortisol (^{124}-I) radioimmunoassay kit (New England Nuclear) in the one and four day groups. Cortical benzodiazepine receptor binding was determined in all subjects using [^{3}H]-flunitrazepam in the presence or absence of clonazepam. Muscarinic cholinergic receptor binding was also studied using [^{3}H]-quinuclidinyl benzilate (QNB) in the presence or absence of atropine sulfate.

Both the SHCS and SH groups exposed to the shock procedure for one four-hour session had elevated levels of corticosterone compared to the CS and HC group. Four days of exposure to the shock contingencies resulted in a decreased level of corticosterone for the SHCS Group. However, corticosterone levels remained elevated in the SH group. Cortical benzodiazepine receptor binding was considerably decreased in both the four-day SHCS and SH group. A moderate decrease was observed in the one-day SH group. Cortical benzodiazepine receptor binding was not altered in the one-day CS, four-day CS, HD or HDC groups. QNB binding in the hippocampus was not altered by any condition studied. These data also suggest that the level of corticosterone, a generally accepted indicator of stress, does not correlate with changes in benzodiazepine or QNB receptor binding. (Supported in part by USPHS Grant DA05252).

326.12 PLASMA BETA-ENDORPHIN AND BETA-LIPOTROPIN RESPONSE TO CONDITIONED STIMULI. B.N. Bunnell, W.E. Hills*, D. Terrell*, J.H. Tigges*, E.H. Mougey*, L.L. Pennington*, and J.L. Meyerhoff. Dept. Psychology, U. Georgia, Athens, GA 30602 & Dept. Med. Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307.

We have previously shown that plasma levels of beta-endorphin (β-EP) are increased in rats by the psychological stress of merely being returned to a chamber where they had received footshock (FS) on preceeding days (Meyerhoff, et al, Neurosci. Abst. 7: 166, 1981). The present study examines whether such biochemical changes can be brought under the control of one specific stimulus by pairing that stimulus with FS. Also, the assay was modified to permit measurement of both β-EP and beta-lipotropin (β-LPH). Five groups of adult female hooded rats were used. Groups A & B received 14 days of identical training during which a light stimulus (L) was paired 20 times daily with FS, on a variable time, 1 min schedule. On each trial, a change in illumination (L) began 8 sec before onset of 5 sec of 1 mA FS; L and FS terminated simultaneously. Groups C & D received 14 days of either FS or L, respectively (see table). Group E was merely handled daily. On the test day, groups A thru D were placed in the shock chamber for 10 min. Group A received only L, while group B received neither L nor FS. Groups C & D received 10 min of FS or L, respectively. Immediately following the test, rats were decapitated and trunk blood collected. Following separation on PrepPak-500/C18 adsorbent, samples were assayed for β-EP and β-LPH immunoreactivity. Results are given as pg/ml ± SEM.

Group	N	Training	Test Day	β-EP	β-LPH
A	5	FS L	0 L	436 ± 83	1837 ± 204
B	5	FS L	0 0	307 ± 35	1030 ± 136
C	5	FS 0	FS 0	830 ±142	4477 ± 925
D	5	0 L	0 L	235 ± 52	979 ± 128
E	3	0 0	0 0	164 ± 23	591 ± 132

Levels of β-EP in groups A & B were higher than in group E, confirming our previous report that psychological stress increases β-EP. Stimulus control of the β-LPH response was shown by the significantly higher β-LPH in group A than in group B. These groups were not significantly different from each other with respect to β-EP. Further extension of these studies are in progress.

327.1 ENHANCED PHOSPHORYLATION OF TYROSINE HYDROXYLASE AT MORE THAN ONE SITE IS INDUCED BY 56 mM K^+ IN RAT PHEOCHROMOCYTOMA PC-12 CELLS IN CULTURE. N. Yanagihara*, A.W. Tank*, T.A. Langan* and N. Weiner. Dept. Pharmacology, Univ. Colo. Health Sci. Center, Denver, CO 80262.

Incubation of rat pheochromocytoma PC-12 cells with 56 mM K^+ is associated with an increase in the activity and enhanced phosphorylation of tyrosine hydroxylase (TH) in the cells. Both the activation and increased phosphorylation of TH are dependent upon the potassium concentration in the incubation medium and on the presence of extracellular calcium (Fed. Proc. 42: 378, 1983). In the studies reported here, we have examined the mechanism of activation and phosphorylation of TH elicited by 56 mM K^+ in rat pheochromocytoma PC-12 cells in culture.

For measurement of the phosphorylation of TH, the cells were preincubated for 30 min with ^{32}P-phosphate (0.5 mCi/ml) in order to label the ATP stores. Tyrosine hydroxylase was purified by immunoprecipitation and subjected to SDS -polyacrylamide gel electrophoresis. An autoradiograph was produced from the gel. The ^{32}P-phosphate incorporated into TH was measured by assessing the density of the autoradiographic band and counting of the eluted TH band by liquid scintillation spectrometry. Tyrosine hydroxylase activity was assayed by the coupled decarboxylase assay after passing the supernatant enzyme through a Sephadex G-25 column.

Incubation of the PC-12 cells with either dibutyryl cyclic AMP (dB cAMP) or 56 mM K^+ is associated with an increase in the activity of TH. These treatments also result in enhanced phosphorylation of TH. Following elution of the ^{32}P-phosphorylated enzyme from the SDS-polyacrylamide gel, and oxidation with performic acid and tryptic digestion of the enzyme, the digest was subjected to paper electrophoresis at pH 1.9, and an autoradiograph of the paper was generated. Two distinct ^{32}P-phosphopeptides are observed. 56 mM K^+ increases ^{32}P-phosphate incorporation into both of these peptides, whereas dB-cAMP increases ^{32}P-phosphate incorporation into one of these peptides. In control studies, employing purified pheochromocytoma TH and catalytic subunit of cAMP-dependent protein kinase, we have shown that only the latter peptide is phosphorylated by cAMP-dependent protein kinase in vitro.

From these results, we conclude that TH in rat pheochromocytoma PC-12 cells is phosphorylated at two or more distinct sites when the cells are depolarized by 56 mM K^+, presumably consequent to the activation of both cAMP dependent and cAMP independent protein kinases. Supported by USPHS grants NS 07927, NS 09199 and AG 03923

327.2 ROLE OF NUCLEAR PHOSPHORYLATION IN THE CONTROL OF PROENKEPHALIN AND TYROSINE HYDROXYLASE (TH) GENE EXPRESSION IN ADRENAL CHROMAFFIN CELLS. H. Kageyama*, J.P. Schwartz, E. Costa and A. Guidotti, Lab. Preclinical Pharmacology, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032

Persistent activation of the splanchnic axon terminals innervating adrenal chromaffin cells induces TH. Here we report that in rat, the transsynaptic induction of adrenal TH by reserpine is paralleled by an increased content of enkephalin-like peptides (ELP) which colocalized with catecholamines in the chromaffin granules. Both the TH induction and the increase of ELP is prevented by transection of the splanchnic nerve. To study the molecular mechanisms involved in this transsynaptic increase of TH and ELP we used primary cultures of bovine adrenal chromaffin cells. Exposure of the cells to 8-Br cyclic AMP (8-Br-cAMP) produces 48 hours later a 2-3 fold increase of ELP and TH. The increase in both parameters was related to the dose of 8-Br-cAMP (from 0.1 to 1 mM) and was not observed after massive doses of 8-Br-cGMP. The increase of TH activity was due to an increased number of TH molecules. The delayed increase of ELP induced by 8-Br-cAMP was associated with an increase of proenkephalin mRNA levels as revealed by hybridization of mRNA with cDNA probe for proenkephalin (Comb et al., Nature 295:663, 1982). Both increases of TH and ELP were blocked by actinomycin D and cyclo-eximide treatment. The addition of 8-Br-cAMP to the cells activates cytosol cAMP dependent protein kinase, whose catalytic subunit translocates from cytosol to the nucleus and increases nuclear protein phosphorylation, and specific poly A^+ RNA messengers. Blockade of protein kinase translocation with colchicine blocks TH induction and the 8-Br-cAMP-induced increase in content of ELP. This suggests that after 8-Br-cAMP both TH and proenkephalin gene expression depends on the translocation of cytosol protein kinase catalytic subunit to the nucleus and nuclear protein phosphorylation.

327.3 QUINOLINIC ACID PHOSPHORIBOSYLTRANSFERASE IN RAT BRAIN. R. Schwarcz, A.C. Foster and K. Iwai*. Maryland Psychiatric Research Center, Baltimore, MD 21228 and Department of Food Science and Technology, University of Kyoto, Kyoto, Japan.

Mammalian quinolinic acid phosphoribosyltransferase (QPRTase; E.C. 2.4.2.19), an enzyme catalyzing the conversion of quinolinic acid (QUIN) to nicotinic acid mononucleotide (NMN), has been extensively desribed in peripheral organs (cf. BBA 611, 280, 1980). Because of the potential relevance of QUIN as an etiological factor in neurodegenerative disorders (Science 214, 318, 1983), we have now investigated the characteristics of QPRTase in rat brain.

For routine analysis, each assay tube contained an aliquot of whole forebrain homogenate (8 mg tissue), 1 mM phosphoribosylpyrophosphate (PRPP), 1 mM $MgCl_2$ and 10 mM 2-mercaptoethanol in 0.5 ml 50 mM potassium phosphate buffer, pH. 6.6. Boiled tissue was used to obtain blank values. The reaction was started by the addition of 10 pmol (0.1 µCi) ^{3}H-QUIN (7.8 Ci/mmol; Nuclear Research Center, Negev, Israel). After incubation for 2 hr at 37°C, tissue was removed by centrifugation and the supernatant applied to a Dowex AG 1X8 (formate form) anion exchange column. ^{3}H-NMN was eluted by successive washes with 0.2M and 0.4M formic acid and radioactivity determined. The identity of ^{3}H-NMN was confirmed in separate experiments by HPLC and TLC techniques.

Enzyme activity was linear with time (up to 2 hr) and tissue amount (up to 16 mg per tube), was optimal at pH 6.6 and entirely dependent on the presence of both PRPP and Mg^{2+}. Kinetic analyses revealed a K_m of 2.3 µM for QUIN and a v_{max} of 8.1 pmol NMN formed/mg tissue/hr; the K_m for PRPP was 1.3 mM. A number of structural analogs and isomers of QUIN, kynurenines and excitotoxins did not inhibit QPRTase activity by more than 30% at 100 µM. Phthalic acid, a potent competitive inhibitor of peripheral QPRTase, powerfully blocked the brain enzyme ($K_i \sim 2$ µM). Subcellular fractionation experiments indicated a preferential localization of QPRTase in synaptosomes. Between different CNS regions, an approx. 10-fold variation in QPRTase activity was observed: olfactory bulb >>hypothalamus>thalamus, substantia nigra>cerebellum, medulla, amygdala, spinal cord>frontal cortex, striatum, hippocampus. Striatal QPRTase activity was clearly demonstrable as early as two days post natum. In the adult striatum, no reduction in the activity of the enzyme was observed 10 days after the local injection of 10 nmol kainic acid (N=5).

A detailed examination of brain QPRTase activity in relation to a possible role of QUIN in neurodegenerative disorders is indicated.

Supported by USPHS grants NS 16102 and 16941.

327.4 TRANSMITTER-RELATED METABOLIC STUDIES IN PURIFIED MOUSE BRAIN SYNAPTOSOMES. Jeffery L. Johnson+ and Eugene Roberts. Div. of Neurosci., City of Hope Research Institute, Duarte, CA 91010.

In nerve terminals, glutamate (Glu) may serve as precursor of the inhibitory neurotransmitter, GABA, and the putative excitatory transmitter, aspartate (Asp), in addition to exerting its own excitatory neurotransmitter role in brain. Glu carbon can originate from glucose through glycolysis and the Krebs cycle, from glutamine (Gln) subsequent to uptake, and from proline (Pro) and ornithine (Orn) (Yoneda et al., J. Neurochem. 38:1686 (1982)). Orn, but not Glu, is an effective precursor of Pro, a putative inhibitory neurotransmitter (Yoneda and Roberts, Brain Res. 239:479 (1982)). We now show that ^{3}H-Arg is converted to Orn in sonicates of purified mouse brain synaptosomes or in intact synaptosomes after uptake, and that the Orn gives rise to Glu, Pro, and GABA. The conversion to other amino acids of labeled Glu, Gln, and Pro subsequent to uptake was studied in subfractionated synaptosomes (fractions 1-4), which layered, respectively, on 1.0 M, 1.2 M, 1.3 M, and 1.5 M sucrose after centrifugation in a discontinuous gradient. Fraction 1 contained small synaptosomal fragments with vesicles and almost no mitochondria. Fractions 2-4 showed numerous mitochondria-containing synaptosomes, fraction 4 containing a unique class of large synaptosomes and more free mitochondria than the other fractions. Glu was readily taken up in all fractions and converted to Asp, Gln, and GABA, the greatest formation of Asp from Glu occurring in fractions 2 and 3 and of Gln in fraction 4. In contrast, Gln was taken up poorly in fraction 1 and not metabolized, converted extensively to Glu and GABA in fractions 2-4, giving rise only to very small amounts of Asp in fractions 2 and 3. Although Pro was taken up to the greatest extent in fraction 2, it was by far most readily converted to Glu, Gln, and GABA in fraction 1, showing only small amounts of Asp formation in fractions 1-3 and none in 4. There was no significant production of Pro from Glu or Gln or of Arg and Orn from any of the three precursors studied. The above results suggest that Glu, Gln, and Pro may be taken up largely in different classes of synaptosomes which are distributed among the centrifugally separation fractions and which possess differing transport and metabolic characteristics. Determination of glutamate decarboxylate activity supplemented the metabolic findings which indicated that GABA-forming nerve terminals were present in all synaptosomal fractions studied.

Supported by grants NS18858 and NS18859 and by a grant to Jeffery L. Johnson from the University of South Dakota.

+Present address: Dept. Physiol. and Pharmacol., The University of South Dakota School of Medicine, Vermillion, SD 57069.

327.5 MULTIPLE FORMS OF CATECHOL-O-METHYLTRANSFERASE IN RAT TISSUES DETERMINED BY GEL ELECTROPHORESIS AND IMMUNE FIXATION. M.H. Grossman[1], M. Braverman[1]*, C.R. Creveling[2], R. Rybczynski[1]*, C. Isersky[2]* and X.O. Breakefield[1]. [1]Dept. Human Genetics, Yale Univ. Sch. Med., New Haven CT 06510; [2]Lab. Bioorganic Chem., NIH-NIAMDD, Bethesda MD 20205.

Catechol-O-methyltransferase (COMT: EC 2.1.1.6) catalyzes the transfer of the methyl group from the donor S-adenosyl-L-methionine, to a phenolic hydroxyl group on catechol steroids, catecholamine neurotransmitters, drugs and many xenobiotic catechols. Several studies have suggested multiple forms of this enzyme, including membrane-bound and soluble forms, and forms differing in apparent MW. Here, COMT and COMT-related proteins were visualized in various rat tissues (liver, heart, pituitary, cerebellum, cerebral cortex, area postrema, kidney, adrenal, hypothalmus, and raphe) utilizing gel electrophoresis, electroblotting of proteins to nitrocellulose ("Western blotting") and immune fixation with antisera to authentic purified rat liver COMT. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) revealed three immune specific proteins of apparent MW 23,000, 26,000 and 66,000 daltons. Isoelectric focusing (IEF) and two-dimensional gel electrophoresis showed three immunoreactive proteins with isoelectric points 5.1, 5.2 and 5.3, the first corresponding to the 26,000 dalton protein and the other two having the same MW, 23,000 daltons. Examination of the particulate fraction of liver revealed an additional minor immunoreactive protein of pI 6.2. Analyses of these proteins for COMT activity in slices from IEF gels indicate that the pI 5.2 species and possibly one of the other proteins are biologically active. Examination of immunoprecipitated in vitro translation products of rat liver polysomal mRNA by SDS-PAGE revealed that the 23,000 dalton and 66,000 dalton proteins are the primary translation products. Comparisons of the radioactivity incorporated into the major immunoreactive protein (23,000 dalton) and acid precipitable radioactivity incorporated into total protein indicate an approximate abundance of 0.2% for the mRNA coding for COMT, making it a good candidate for mRNA immune isolation and possible cDNA cloning. In conclusion, COMT appears to exist in several forms differing in both charge and molecular weight in all rat tissues examined. Once a cDNA to the mRNA coding for COMT is isolated we will be able to determine the number of genes coding for this enzyme and hence elucidate the basis of its multiplicity.

327.6 EFFECTS OF ENDOGENOUS MODULATORS OF MONOAMINE-OXIDASE ON THE METABOLISM OF SEROTONIN, C.T. Giambalvo and R.E. Becker. R.I. Psychiatric Research & Training Center, University of Rhode Island Brown University, Cranston, R.I. 02920.

We have previously reported the presence of an endogenous modulator of MAO-A in plasma (Life Sciences 29:347, 1981), CSF (Science, in press) and brain tissues (Soc. Neuroscience Abs. 8:818, 1982). This modulator seems to be a trypsin-sensitive peptide of about 4000 in MW. To further access the physiological significance of this modulator, we have examined the effect of the modulator on 5-HT metabolism in synaptosomes in vitro and after intracerebral injections.

The modulator was fractionated through Sephadex-G 50 column chromatography. Aliquots were added to synaptosomes prepared from rat striatum, and incubated for 20 minutes at 37° C. The reaction was terminated by centrifugation. The pellets were extracted for 5-HT and 5-HIAA, which were then analyzed with HPLC with electrochemical detection. The modulator was found to increase the level of 5-HT and decrease that of 5-HIAA. This effect was temperature and time dependent. Increasing the amounts of the modulator also led to increases in the effect observed.

Aliquots of the modulator were also injected unilaterally into the striatum of rats. An inhibition of MAO-A was observed in the injected side compared to the control side of the brain. The injected side also showed an increase in 5-HT levels and a decrease in 5-HIAA levels.

The distribution of the modulator in the brain paralleled that of MAO-A distribution. The highest levels were observed in the Raphe and substantia nigra; the lowest level was found in the pons-medulla.

327.7 DOPAMINERGIC MECHANISM IN THE CONTROL OF MET-ENKEPHALIN RELEASE FROM RAT STRIATAL SLICES. F. Battaini*, S. Govoni, G. Pasinetti*, C. Missale*, P.F. Spano and M. Trabucchi. (SPON: J.G. Krikorian) Dept. of Pharmacology and Pharmacognosy, University of Milan and Dept. of Pharmacology, University of Brescia. Italy.

Met-enkephalin may act as a neuromodulator at dopaminergic synapses. In particular, it has been shown that treatments leading to a chronic impairment of dopamine transmission induce an increase in met-enkephalin immunoreactive material (ME-IR) content in selected dopaminergic nuclei. On the other hand, opiate receptors are localized on striatal dopaminergic terminals and the treatment with opiates can modify dopamine turnover in rat striatum. To gain insight into the interaction between dopamine and enkephalin systems, we studied the mechanisms regulating met-enkephalin release from rat striatal slices. Male Sprague Dawley rats were killed by decapitation, the brains were rapidly removed and the striata dissected out. Striatal slices (350 uM) were incubated in oxygenated Krebs-Ringer; met-enkephalin like peptides released in the medium were measured by radioimmunoassay. The addition of 50 mM K^+ induced an over three fold Calcium dependent increase in ME-IR efflux. The basal release of ME-IR is increased in a dose dependent manner by the in vitro addition of dopamine; the K^+ evoked release of ME-IR is also enhanced by dopamine. The stimulatory action of dopamine is shared by apomorphine but not by norepinephrine. Moreover, neuroleptics do not alter the basal or the K^+ stimulated ME-IR release but reverse the dopamine mediated increase. These results suggest that the stimulation of dopamine receptors may influence enkephalin release within striatum. This mechanism may be of relevance for the fine tuning of dopaminergic terminals activity.

327.8 IS THE NORADRENERGIC INHIBITION OF CORTICAL ACETYLCHOLINE RELEASE GABA-MEDIATED? L. Beani, C. Bianchi*, S. Tanganelli* and T. Antonelli*. Department of Pharmacology, School of Medicine, University of Ferrara, 44100 Ferrara, ITALY.

In previous studies we found that Norepinephrine (NE) as well as Locus Coeruleus Stimulation inhibit Acetylcholine (ACh) release and increase GABA outflow from the guinea-pig neocortex (Beani et al.,Eur.J.Pharmacol.,48, 179, 1978; Moroni et al., Brain Res., 232, 216, 1982). Since GABA inhibits ACh release from electrically-stimulated slices through bicuculline-sensitive receptors (Bianchi et al., Arch. Pharmacol.,318, 253,1982), experiments were undertaken to check whether the noradrenergic inhibition of ACh release is direct or GABA-mediated.

Slices of guinea-pig neocortex were superfused with eserinized saline solution and the outflowing ACh was bio-assayed. The inhibitory effect of exogenous NE on ACh release was tested under normal conditions and after pharmacological manipulation of the GABA system.

Picrotoxin ($3x10^{-5}$mol/l) and Bicuculline (10^{-4}mol/l), which by themselves increased the electrically-evoked ACh release, antagonized the inhibition (about 60%) exerted by NE($3x10^{-5}$mol/l on 1 and 5 Hz-evoked ACh release. Similarly, Diazepam ($3x10^{-6}$ mol/l), which prevented GABA inhibition on ACh release in this brain area, counteracted the effect of NE $3x10^{-5}$mol/l. On the contrary, the GABA re-uptake inhibitor 2,4 diamino butyric acid, which at $3x10^{-5}$mol/l hardly affected ACh release, disclosed a significant inhibition (80%) by NE added at the sub-threshold concentration of $6x10^{-6}$mol/l.

These results may indicate that in the guinea-pig neocortex in vitro, Noradrenaline indirectly affects the cholinergic nerve endings, by increasing the tonic inhibition exerted on them by the intrinsic GABAergic interneurons.

Research supported by C.N.R. Grant, n. 82.02103.04.

327.9 CONTRIBUTION OF THE DORSAL NORADRENERGIC BUNDLE TO THE EFFECT OF AMPHETAMINE ON THE TURNOVER RATE OF ACETYLCHOLINE (TR_{ACH}) IN THE HIPPOCAMPUS AND CORTEX OF THE RAT. S.E. Robinson, D.M. Shamel*, and M.J.F. Austin*. Department of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA 23298.

Amphetamine increases TR_{ACh} in various brain regions by a mechanism involving norepinephrine (Robinson et al, Naunyn-Schmiedeberg's Arch. Pharmacol., 304: 263, 1978). In order to determine the role of the locus coeruleus in this action of amphetamine on cholinergic neurons in these areas, the effect of dorsal noradrenergic bundle lesion was studied on the cholinergic response to amphetamine. TR_{ACh} was used as a measure of cholinergic activity and was determined mass fragmentographically by measuring the incorporation of deuterium into ACh and choline after infusion with deuterated phosphorylcholine. Stereotaxically-placed injections of the neurotoxin 6-hydroxydopamine (2µl of 2µg base/µl in 0.02% ascorbic acid, 0.9% saline) were made bilaterally into the dorsal noradrenergic bundle (AP +0.6, L ± 1.3, V -0.5, according to Konig and Klippel) of Equithesin-anesthetized male Sprague-Dawley rats. Control animals were injected bilaterally with the vehicle in the dorsal noradrenergic bundle. Ten days later TR_{ACh} was determined one hour after injection of amphetamine (27µmol/kg, i.p.) or injection of saline. The specificity of the lesion for noradrenergic neurons was determined by measuring levels of norepinephrine, dopamine, and serotonin by an HPLC technique in aliquots of each sample in which TR_{ACh} was determined. Injection of 6-hydroxydopamine into the dorsal noradrenergic bundle reduces norepinephrine levels in the hippocampus and the cortex to 21% and 22% of control, respectively. Dopamine and serotonin levels in these areas were not significantly changed by the injection of the neurotoxin. In the hippocampus and the cortex, amphetamine significantly increases K_{ACh} and TR_{ACh} without affecting levels of ACh and choline. 6-Hydroxydopamine lesions did not significantly affect ACh levels, K_{ACh}, or TR_{ACh}. However, 6-hydroxydopamine lesions of the dorsal noradrenergic bundle prevent significant increases in hippocampal and cortical K_{ACh} and TR_{ACh} in response to amphetamine. Therefore, the neurons in the dorsal noradrenergic bundle appear to be important in the stimulatory action of amphetamine on TR_{ACh} in the hippocampus and cortex, but do not have a tonic action on cholinergic neurons in these areas. (Supported by NIMH MH37450).

327.10 RECEPTOR-MEDIATED MODULATION OF THE RELEASE OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) FROM CAT CEREBRAL CORTEX. J.-Y. Wang*, T.L. Yaksh* and V.L.W. Go* (SPON: E.H. Lambert). Depts. of Pharmacology, Neurosurgical Research and Gastroenterology, Mayo Foundation, Rochester, MN 55905.

By radioimmunoassay utilizing antisera No. 4823 (Brain Res. 241:279-290, 1982), we have previously shown that VIP-like immunoreactivity (VIP-LI) is concentrated in the cerebral cortex of the cat and the resting release can be measured in vivo by cortical cup superfusion. Chemical stimulation (by potassium or veratridine) as well as electrical stimulation (at cortical surface or in the reticular formation) evoked release several times that measured under resting levels. The stimulated release, but not resting release was blocked by depletion of calcium ions and substitution of EDTA or cobalt. Sephadex gel filtration chromatography (G-50, superfine) showed that VIP-LI in cortical tissue as well as in resting and evoked superfusate co-chromatographed with porcine gut VIP-28 standard. Since the distribution of VIP-containing neurons overlaps that of many putative cortical neurotransmitters, we therefore further explored the synaptology by examining the effect of other neurotransmitters on the resting and evoked release of VIP. Cats were anesthetized with chloralose-urethane and cannulated with a tracheal tube. The cortical cup superfusion consisted of two 1 cm diameter cylinders which were agar-sealed on the pial surface of the exposed cortex after bilateral craniotomy and reflection of the dura. Artificial cerebrospinal fluid (CSF) containing albumin/bacitracin was superfused at a rate of 0.1 ml/min. Naloxone (Nal, 0.2-0.6 mg/kg) and natrexone (Ntx, 2-5 mg/kg) administered intravenously enhanced electrically stimulated release and potassium-evoked release and also slightly elevated spontaneous release. Cortical application of Nal (10^{-5}-10^{-4}M) or Ntx (10^{-4}M) also significantly increased evoked and resting release but the magnitude of the effect was less than that observed after i.v. injection of the drug. Morphine ($5x10^{-5}$M) applied topically suppressed potassium-evoked and MRF stimulated release but not significantly inhibited surface-stimulated release. The effect of Nal or Ntx could be attenuated by morphine and the inhibition of morphine can be reversed by naloxone. Phentolamine (10^{-5}M) enhanced the stimulus-evoked release but the resting release was not affected. GABA suppressed stimulated but not resting release. Picrotoxin (10^{-5}-10^{-4}M) increased both resting release and stimulated release. Cholinergic agonist (carbachol, 10^{-5}-10^{-4}M) and antagonist (atropine, 10^{-4}M) did not significantly affect VIP release. These results indicate that opiate, α-adrenergic and GABA receptors are involved in the modulation of cortical VIP release. (NS06663; Mayo Foundation)

327.11 ADENOSINE A_1 AND $GABA_B$ RECEPTORS MAY SHARE COMMON POST-RECOGNITION SITE ELEMENTS OF THE ADENYLATE CYCLASE COMPLEX. W.J. Wojcik and N.H. Neff, Lab. Preclinical Pharmacology, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032

In a synaptosomal membrane preparation from rat cerebellum, we observed an adenosine A_1 and a $GABA_B$ receptor mediated inhibition of adenylate cyclase activity. For testing possible interactions between these receptor systems, N-6 phenyl(isopropyl)adenosine (PIA), an adenosine analogue, was the agonist for the A_1 receptor, while (-)-baclofen a GABA analog, was the agonist for the $GABA_B$ receptor. From studies with neurologically mutant mice we have determined that both receptors are associated primarily with cerebellar granule cells. We therefore evaluated whether a biochemical interaction existed between these two receptors that mediate inhibition of adenylate cyclase. By comparing dose-response curves for PIA in the presence or absence of various concentrations of baclofen we observed that the curves for inhibition of adenylate cyclase were not parallel or additive, but that the same maximal inhibition occurred with high concentrations of either drug alone or in combination. In contrast with the PIA-baclofen response, additive parallel responses were obtained with adenosine A_1 inhibitory receptors (PIA) and the beta-adrenergic stimulatory receptor (isoproterenol) when activated in homogenates of cerebellum. This is not surprising as they are the consequence of two independent simultaneous events occurring on membranes derived from different cells, adenosine A_1 receptors of granule cells and beta-adrenergic receptors of Purkinje cells. The converging dose-response curves for baclofen and PIA indicate that independent receptor recognition sites occur on the same membrane fragments derived from the same cells. They also indicate a possible common rate-limiting biochemical step in the mechanism by which adenosine A_1 and $GABA_B$ receptors inhibit adenylate cyclase activity.

327.12 VAGAL AFFERENTS AND SEROTONERGIC TERMINALS FORM SYNAPSES WITH CATECHOLAMINERGIC NEURONS IN RAT AREA POSTREMA. V.M. Pickel, J. H-L Chan*, T.H. Joh, and A. Beaudet. Lab of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021 and Dept. of Neuro. and Neurosurgery, Montreal Neurol. Inst., Quebec, Canada

The area postrema (AP) of many species has been shown to contain catecholamines and serotonin and to receive direct input from vagal afferents of the nodose ganglia. We sought to determine: (1) the ultrastructural morphology of the serotonergic terminals and vagal afferents; and (2) the synaptic relation between these afferents and catecholaminergic neurons within the rat AP. These objectives were accomplished by combining electron microscopic radioautography for ^{3}H-5-hydroxytryptamine (^{3}H-5HT) or ^{3}H-amino acids anterogradely transported from the nodose ganglion with the immunocytochemical localization of tyrosine hydroxylase (TH), a specific enzymatic marker for catecholamines. Over a 2h interval, 5 µl of 10^{-4}M ^{3}H-5HT plus 10^{-2}M unlabeled norepinephrine were infused into the lateral ventricle of anesthestized, Pargyline-treated, adult male rats. A second group of rats received unilateral injections into the nodose ganglion with one µl (10 µ Ci) of L-proline (2,3,4,5,-^{3}H) and L-leucine (3,4,5-^{3}H). Immediately after the termination of the ^{3}H-5HT infusion and 3 days after the nodose injection, the brains were fixed by aortic arch perfusion with 4% paraformaldehyde and 0.2% glutaraldehyde, and immunocytochemically labeled with antiserum to TH. By light microscopy, both the ^{3}H-5HT terminals and vagal afferents were heavily localized to the dorsal and ventrolateral regions of the AP. In contrast, the TH labeled perikarya and processes were found throughout the AP. Electron microscopic analysis of the dorsal and lateral regions of the AP revealed that the silver grains for ^{3}H-5HT were localized in axon terminals. These terminals contained small, clear and 1 or more dense core vesicles and measure 0.5 - 2 µm in diameter. They formed symmetric synapses with both TH-labeled and unlabeled dendrites. The 5HT terminals were also present within the perivascular and ventricular spaces. As compared with 5HT terminals, vagal afferents were larger (2-3 µm), contained more numerous dense core vesicles and similarly formed synapses with both TH labeled and unlabeled dendrites within the AP. These studies demonstrate that primary afferents and serotonergic terminals make monosynaptic synapses with catecholaminergic neurons in the rat AP. The synaptic relations are the same as previously reported in the nucleus tractus solitarius and thus suggest related functions for the AP and NTS.
(Supported by Grants HL18974 and MH00078).

327.13 ANATOMICAL EVIDENCE FOR INTERACTIONS BETWEEN CATECHOLAMINE AND ACTH-CONTAINING NEURONS. S. Alden*, H. Baker, D.A. Ruggiero, M. Anwar* and D.J. Reis (SPON. A.B. Judd). Lab or Neurobiol., Cornell Univ. Med. Coll., New York, NY 10021

Recent studies suggested a close correspondence between the distribution of neurons containing tyrosine hydroxylase (TH) and ACTH in ventral hypothalamus. We sought to investigate whether the same neurons contain both TH and ACTH. In colchicine treated or untreated rats antigens were localized using specific antibodies and the PAP technique with 4-chloronaphthol (4ClNa) or diamino benzidine (DAB) as chromagens. Experimental protocols employed were: (1) Adjacent sections stained with TH or ACTH antibodies; (2) Single sections stained with ACTH (with 4ClNa) photographed, and chromagen and antibody removed. Sections restained with TH (with DAB), rephotographed and compared; (3) Single sections stained sequentially with both antibodies and chromagens; without removing the first antibody or chromagen. Data obtained from adjacent sections demonstrated that at midlevels of the arcuate nucleus (Arc) cells containing ACTH and TH formed overlapping hyperbolic lamina. Little or no overlap occurred in dorsal periventricular aspects of Arc while at mid to caudal tuberal regions, the ventrolateral expansion of the TH group resulted in marked overlap of ACTH and TH immunoreactive neurons. Although located in the same regions, few, if any, double labeled cells were found even though two labeling methods were used to identify them. However, ACTH and TH neurons often formed small clusters of apposed cells. In paraventricular, medial and posterior hypothalamic nuclei, adjacent sections also showed a close correspondence between ACTH terminals and TH cell bodies. ACTH terminals were not prominent in Arc but those present did overlap regions containing TH perikarya. Overlap also occurred between TH and ACTH terminals in structures including organum vasculosum lamina terminalis, bed nucleus of stria terminalis and paraventricular thalamus. In contrast, other regions displayed striking compartmentalization of TH and ACTH terminal fields; e.g., in both the paraventricular hypothalamic nucleus and central n. of amygdala, ACTH was located ventrally, while TH was localized to more dorsal aspects of the nuclei. We conclude: (1) TH and ACTH immunoreactivity are present in similar regions of Arc; (2) the two antigens are not contained in the same neurons; (3) terminal fields overlap in some regions but are separated in others; (4) ACTH terminals overlap TH containing cell bodies in many regions. These data provide anatomical support for a functional interaction between catecholamine and ACTH systems in restricted areas of hypothalamus and forebrain. (Supported by Grant MH 33190).

327.14 COLORIMETRIC ASSAY FOR HUMAN PLATELET MAO USING PEROXIDASE/ABTS AS A MARKER SYSTEM. A. Szutowicz*, P.J. Orsulak* and R.D. Kobes* (SPON: W. G. Clark) Department of Psychiatry, Univ. of Texas Health Science Center and VAMC, Dallas, Texas 75235

A non-radiometric method for the determination of monoamine oxidase (MAO, monoamine: oxygen oxidoreductase (deaminating), EC 1.4.3.4.) activity in human platelets has been developed. MAO activity in platelets is assayed by a colorimetric method using horse radish peroxidase and 2,2'-azino-di-(3-ethylbenzthiazoline sulfonic acid) (ABTS) to determine the amount of H_2O_2 formed during the oxidation of tyramine by the enzyme. The assay medium contains: 100mM sodium phosphate buffer (pH 7.4), 1.0mM tyramine and 6.2mM sodium azide in a final volume of 0.5ml. Blanks do not contain tyramine. The reaction is started by addition of 0.2mg of platelet protein and incubation is continued for 20 min at 37°C. The reaction is stopped by addition of a 0.5ml of H_2O_2 measuring solution containing 1.8mM ABTS and 5U of peroxidase in 500mM phosphate/citrate buffer (pH 4.0) followed by addition after 15 sec of 0.25ml of 0.75M HCl with 5% sodium dodecyl sulphate. The colored product is read at 414nm within 60 minutes after the assay. The reaction is linear up to 30 minutes and the amount of H_2O_2 found is proportional to the amount of platelet protein within the range of 0.1 to 0.5mg per assay. The recovery of 4nmols of exogenous H_2O_2 added to the assay varied from 94 to 83% after 20 minutes incubation with 0.2 and 0.5mg of platelet protein, respectively. The addition of pargyline caused 98% inhibition of H_2O_2 formation in the assay system. Intra-assay coefficients of variation were 1.5 and 2% for the colorimetric and radiometric assays, respectively. Specific activity of MAO in platelets isolated from blood of people without psychiatric disease, with tyramine as a substrate was found to be 0.46 ± 0.10 and 0.37 ± 0.06nmols/min/mg of protein for the colorimetric and radiometric assays, respectively. Values reported are within the range of activities found in the literature. Although values of MAO activity obtained by colorimetric method are 20% higher than those found with the radiometric method, the correlation between these two methods is significant (r=0.918, p 0.001). These data indicate that the method is specific and applicable for the determination of MAO activity in clinically obtainable human platelets.

OPIATES, ENDORPHINS, AND ENKEPHALINS: PHYSIOLOGICAL EFFECTS IV

328.1 IONTOPHORETIC APPLICATION OF MORPHINE AND NALOXONE ON VENTRO_ MEDIAL HYPOTHALAMIC UNITS. B. Prieto-Gómez*, C. Reyes-Vázquez and N. Dafny. Depto. Fisiol. Fac. Med. UNAM. México and Dept. Neurobiol. UT Med. Sch. Houston, Tx. 77025.

Previous studies using systemic application of morphine (MOR) in freely behaving animals implanted with permanent semi-microelectrodes within the ventromedial hypothalamus (VMH) demonstrated significant changes in neuronal discharges. Since systemic MOR administration may affect remote structures which can influence the VMH, the present study was initiated to determine the effect of the microiontophoretically applied MOR on the spontaneous activity of VMH units. In urethane anesthetized rats after craniectomy an array of 5 micropipettes was lowered within the VMH. The micropipettes for local injection contained: (1)MOR 0.043M pH=4.5; (2)naloxone (NAL) 0.01M pH=5.0; (3)L-glutamate 0.01M pH=8.0; (4)fast green in NaCl 3M (balance and test current) and (5)the recording electrode filled with NaCl 3M. Fifty units were tested with MOR (5, 20 and 50 nA), NAL alone (50 nA) and with simultaneous application of MOR (50 nA) and NAL (50 nA). Glutamate (5-50 nA) and current effects (100 nA) were tested in each cell for control purposes. Fifty-eight % of VMH units failed to respond to local MOR application. In those cells affected by MOR 75% and 25% exhibited decreases and increases in firing rates respectively. The MOR responsive cells demonstrated a dose-related response. NAL applied simultaneously with MOR had an antagonistic effect only in the units in which MOR induced a decrease of electrical discharge. However, in some units and additive effect by the simultaneous application of both drugs was observed. Several units responded to NAL applied alone by a decrease (25.9%) or an increase (11.1%) in their electrical discharges and 69% of the units failed to modify their activity following NAL ejection. Glutamate produced an excitation on all the units recorded and no cell was affected by current ejection. Our observations indicate that VMH has a low sensitivity to MOR applied iontophoretically as compared to previous experiments using the systemic route. This observation could be the result of a low density of μ receptors as has been suggested by neurochemical studies. The MOR depressive effects seem to be specific, since these could be blocked by NAL, while the NAL-resistant excitatory actions of MOR could represent some nonspecific effects of the drug. The effects of NAL alone, as well as its additive action when applied with MOR, showed by some units, suggest that NAL is not necessarily a pure antagonist in VMH. It is possible that the effects observed in VMH by the systemic application of MOR are mediated through other structures, although the direct MOR action observed in the present study could contribute to such effects.

328.2 EXTRACELLULAR CALCIUM TRANSIENTS FOLLOWING SPINAL TRAUMA: DECREASED RECOVERY TIME FOLLOWING NALOXONE ADMINISTRATION. G.E. Hollinden* and B.T. Stokes. Dept. of Physiology, The Ohio State University School of Medicine, Columbus, Ohio 43210.

The extracellular concentration of calcium ion was measured in canine spinal cord subsequent to spinal injury. Calcium sensitive microelectrodes were used to characterize interstitial calcium activities at single loci in the canine spinal cord. In the control animal, we found that calcium activities changed little independent of electrode placement in the spinal cord, were stable during the 3 h necessary to make injury measurements, and were comparable to other estimates of calcium in the interstitial space. All animals were injured using a modified Allen drop technique that leaves chronic animals permanently paraplegic. After injury, calcium activities decreased to micromolar levels that were incompatible with neural function. An incomplete recovery of extracellular calcium occurred during the next 3 h to about one-third (0.44 ± 0.01 mM) of the normal value (1.1 ± 0.08 mM). Such a pattern of changes in extracellular calcium was specific for the injury site itself and did not occur at nearby anatomic loci. If a bolus (10 mg/kg) of naloxone hydrochloride (ENDO Labs) was given at twenty minutes post-injury, calcium activities increased significantly above the passive recovery curve at 1 hr. post-naloxone. Complete recovery of extracellular calcium occurs within the three hour period monitored. These results are interpreted as having both short- and long-term effects on neuronal function and subsequent reorganization of spinal pathways. Supported by USPHS NIH grant NS-10165.

328.3 DYNORPHIN DECREASES CALCIUM CONDUCTANCE OF MOUSE CULTURED DORSAL ROOT GANGLION NEURONS. R.L. Macdonald and M.A. Werz. Dept. of Neurology, University of Michigan, Ann Arbor, MI 48109.

Previously we reported that both mu- and delta-opiate receptors are present on dorsal root ganglion neuron (DRG) somata, that these receptors have a heterogeneous distribution among DRG neurons, and that both receptor types decrease the duration of somatic calcium-dependent action potentials (Werz and Macdonald, Nature 299:730, 1982). Therefore, these opiate receptors might have a function similar to those on primary afferent terminals where a decrease in calcium entry would be correlated with a decrease in transmitter release. Additionally, we have suggested that both mu- and delta-opiate receptors are coupled to potassium channels and that activation of these receptors results in an augmentation of a potassium conductance (see Werz and Macdonald, Neurosci. Abstr. 1983). The purpose of the present investigation was to compare the actions of the opioid peptide, dynorphin, which may preferentially bind to kappa-receptors, with the action of morphiceptin, a mu-receptor selective ligand, and leucine-enkephalin, a delta-receptor preferring ligand.

Cell culture and intracellular recording techniques were as previously described (Werz and Macdonald, Brain Res. 239:318, 1982). Opioid peptides were applied by pressure ejection from micropipettes with tip diameters of 2-5 μm.

We report that dynorphin at 100 nM to 1 μM decreased somatic calcium-dependent action potential duration in a portion of DRG neurons and that dynorphin action was antagonized by naloxone. DRG neuron responses to dynorphin differed from responses to morphiceptin or leucine-enkephalin. Firstly, many DRG neurons responded to dynorphin but not to morphiceptin or leucine-enkephalin. Secondly, dynorphin responses, unlike morphiceptin or leucine-enkephalin responses, persisted after intracellular injection of the potassium channel blocker, cesium. We suggest that dynorphin acts at a receptor distinct from mu- and delta-receptors (possibly kappa-receptors), that this receptor may be distributed on DRG neurons differently than mu- or delta-receptors, and that this receptor is coupled to a voltage-dependent calcium channel.

Supported by BNS18762 and DA05244.

328.4 OPIOID PEPTIDES SELECTIVE FOR MU- AND DELTA-OPIATE RECEPTORS INCREASE A DRG NEURON POTASSIUM CONDUCTANCE. M. A. Werz and R. L. Macdonald, Dept. of Neurology, University of Michigan, Ann Arbor, MI 48109.

Opioid peptides decrease somatic calcium-dependent action potentials of mouse dorsal root ganglion (DRG) neurons grown in cell culture. We have recently reported that opioid peptides selective for both mu- and delta-opiate receptors decrease the duration of DRG neuron calcium-dependent action potentials (Werz and Macdonald, Nature 299: 730, 1982). We based our conclusion on the findings of a heterogeneous response of DRG neurons to the mu-receptor selective ligand morphiceptin and the delta-receptor preferring ligand leucine-enkephalin as well as a differential sensitivity to naloxone. The purpose of the present investigation was to identify the ion channels to which mu- and delta-receptors are coupled.

Cell culture and intracellular recording techniques were as previously described (Werz and Macdonald, Brain Res.239: 318, 1982). Opioid peptides were applied to single neurons by pressure ejection from micropipettes with tip diameter of 2-5 μm. Opioid peptide effects were first assessed during intracellular recording with potassium acetate (KAc)-filled micropipettes. The KAc-filled recording micropipette was then gently withdrawn, the neuron was reimpaled with a recording micropipette containing cesium acetate (CsAc) and cesium, a potassium channel blocker, was injected intracellularly. The action of the opioid peptides was then reassessed on the prolonged calcium-dependent action potentials following cesium injection.

We now suggest that both mu- and delta-receptors are coupled to voltage- and/or calcium-dependent potassium channels since opioid peptide decreases of calcium-dependent action potentials were associated with: 1) an absence of effect on resting membrane potential or conductance, 2) an increased action potential after-hyperpolarization, and 3) blockade of opioid peptide action by intracellular injection of cesium. In contrast, norepinephrine and cadmium, which have been reported to decrease calcium-dependent action potential duration by an action on voltage-dependent calcium conductance, decreased the amplitude of the action potential after-hyperpolarization and decreased calcium-dependent action potential duration following intracellular iontophoresis of cesium.

Supported by BNS18762 and DA05244.

328.5 EFFECTS OF SYSTEMIC MORPHINE UPON HIPPOCAMPAL ELECTROPHYSIOLOGY IN THE RAT. G. Rose*, K. Pang* and T. Dunwiddie, Dept. of Pharmacology, UCHSC and Medical Research, VMAC, Denver, CO.

Opiates have been shown to profoundly increase the excitability of hippocampal pyramidal cells. A suggested mechanism for this unusual excitatory action is via a reduction in local-circuit inhibition, mediated by an opiate-specific inhibition of the firing of inhibitory interneurons. Hippocampal neurons have been classified into two types, complex-spike cells and theta cells, which differ from each other in both their electrophysiological characteristics and behavioral correlates. Theta cells in the hippocampus proper have been tentatively identified as interneurons. The present study examined the effects of systemic morphine injections upon the activity of these two hippocampal cell types.

Recordings were made from the dorsal hippocampal CA-1 pyramidal cell layer of either unanesthetized, freely moving rats or from urethane-anesthetized preparations. A stimulating electrode was placed in the ventral psalterium to activate commissural afferents. Respiration was monitored in the anesthestized animals to provide a physiological index of the drug response. Intraperitoneal injections of 7.5 mg/kg morphine sulfate elicited overt behavioral effects (pronounced akinesia, tail-pinch analgesia) in the freely moving rats. However, at this dose the population spike evoked by commissural stimulation was not affected; 10 mg/kg was necessary to produce significant changes in population spike amplitude. The activity of both complex-spike and theta cells was consistently elevated at both doses, although for the theta neurons short periods of depression preceeded excitation. In the anesthetized animals, 15 mg/kg elicited mixed effects upon the population spike. Most cases showed increased amplitude, but this was sometimes preceeded by depression; depression alone was seen in approximately 30% of the experiments. Regardless of the effect of morphine on the population spike, both complex-spike and theta cells showed increased spontaneous activity, often with pronounced bursting. Theta cells again showed transient depression preceeding the excitation. All effects were reversed by naloxone (2 mg/kg).

In summary, behavioral effects of morphine were seen at doses which affected unit activity, but not the population spike evoked by commissural stimulation. At higher doses effects upon the population spike were bidirectional and sometimes biphasic, whereas the spontaneous activity of both complex-spike and theta cells always increased. This data suggests that the predominant opiate effect which has been described in the hippocampus in vitro (viz., increase in population spike amplitude) does not necessarily occur in vivo with behaviorally relevant doses of morphine.

328.6 HYPERPOLARIZATION OF SUBSTANTIA GELATINOSA NEURONS IN VITRO BY ENKEPHALIN AND NORADRENALINE. M. Yoshimura and R. A. North Neuropharmacology Laboratory, M.I.T. 56-245, Cambridge, MA 02139

Enkephalin containing terminals and cell bodies and also catecholamine reactive terminals have been shown by immunohistochemistry to be abundant in the substantia gelatinosa (SG) of the spinal cord. We have investigated the effects of enkephalin and noradrenaline on SG neurons by intracellular recording. A lumbosacral laminectomy was performed on adult rats anesthetized with urethane (1.5 mg/kg). After removal of pia and arachnoid from a 1.5 cm length of spinal cord, serial transverse slices were cut with a vibratome (4-6°C) at a thickness of 400 μm. A slice then was continuously superfused with a physiological saline solution at 37°C. Intracellular recordings were made with 3 M KCl filled electrode (DC tip resistance of 60 - 120 MΩ). Slices were viable for up to 24 hours, and single cell recordings were typically several hours.

SG cells had resting membrane potential of -60 - -70 mV and input resistance of 100 - 400 MΩ. Superfusion with normorphine (1 - 10 μM) and the enkephalin analog D-Ala^2-D-Leu^5-enkephalin (100 nM - 2 μM), produced concentration dependent hyperpolarizations associated with an increased membrane conductance in about 50% of the SG cells tested. Noradrenaline (1 - 50 μM) caused a membrane hyperpolarization associated with conductance increase in 80% of the cells. Similar effects were obtained using pressure application to a micropipette containing Met^5-enkephalin or noradrenaline with its tip positioned in the solution above the slice. The hyperpolarization became smaller when the membrane potential was shifted to the potassium equilibrium potential and eventually reversed in polarity. The reversal potential followed the external potassium concentration as expected from the Nernst equation. Naloxone (3 - 300 nM) inhibited enkephalin responses. The noradrenaline hyperpolarizations were depressed by phentolamine (1 μM) but not by propranolol (1 μM). α_2-adrenoceptors are probably involved since yohimibine (500 nM) antagonized the response but prazosin (1 μM) did not. These enkephalin and noradrenaline induced hyperpolarizations were direct postsynaptic actions because when the neurons were superfused with solutions containing 2 mM CO/20 mM Mg or 0.25 mM Ca/5 mM Mg, these responses persisted although evoked synaptic potentials disappeared. In conclusion, opiate drugs and noradrenaline interact with a naloxone sensitive receptor and α_2-adrenoceptor, respectively, and both result in hyperpolarization by increasing potassium conductance. These effects are appropriate to substances known to suppress the transmission of pain through the SG.

328.7 OPIATE ACTIVATION OF G_K INHIBITS CALCIUM SPIKES IN RAT LOCUS COERULEUS. J. T. Williams and R. A. North, Neuropharmacology Laboratory, M.I.T. 56-245, Cambridge, MA 02139

Opiates inhibit the firing and hyperpolarize locus coeruleus (LC) neurons by opening potassium ion channels. The release of noradrenaline from cortex and cerebellum evoked by high potassium solutions is also depressed by opiates. Since the majority of noradrenergic terminals in these structures arise from neurons in the LC, we hypothesized that the potassium activation could account for both the inhibition in firing and the depression of transmitter release. We made intracellular recordings from rat LC neurons in the slice preparation. Slices of pons containing the LC were maintained completely submerged in artificial cerebrospinal fluid of the following composition (mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2.4, NaH_2CO_3 25, glucose 11, gassed with 95% O_2 5% CO_2 at 37°C. Drugs were applied by pressure ejection and by superfusion. The action potentials of LC neurons involve both sodium and calcium entry. We studied the calcium action potential in the presence of tetrodotoxin (TTX); this spike was dependent on the extracellular concentration of calcium ions and was reversibly abolished by cobalt. Opiates (1 - 10 μM) still hyperpolarized LC neurons in TTX; the spike was studied after resetting the membrane potential to its control level with depolarizing current. Opiates decreased the rate of rise and amplitude of the calcium action potential and decreased the afterhyperpolarization following the spike. The reduction of the calcium spike by opiates was greatest in neurons which had more slowly rising and lower amplitude action potentials. When the potassium activation by opiates was blocked by barium (30 μM - 1 mM) applied extracellularly or caesium applied intracellularly opiates did not decrease the duration of the calcium spike. Opiates also did not depress a voltage-dependent persistent inward calcium current measured under voltage clamp (Dagan single electrode clamp).

An inhibitory postsynaptic potential (IPSP) recorded in LC neurons is mediated by noradrenaline probably released from recurrent collaterals or dendro-dendritic contacts between LC neurons. We used the IPSP as a measure of noradrenaline release. Opiates reduced the amplitude and duration of the IPSP while having little or no effect on hyperpolarizations induced by exogenous noradrenaline. These results can be interpreted on the assumption that the action of opiates close to the region of LC neurons releasing the noradrenaline is the same as that in the cell body. In this case, the inhibition of release may result from the potassium activation. Two possible mechanisms for this are a block of action potential propagation and the indirect reduction in calcium entry.

328.8 HIPPOCAMPAL NEURONS IN PRIMARY CULTURE: PATCH CLAMP AND IMMUNOHISTOCHEMICAL STUDIES. Daniel B. Hoch*, Elizabeth Bostock, Anita A. Roth* and Raymond Dingledine. Dept. Pharmacology and Neurobiol. Curric., Univ. North Carolina, Chapel Hill, NC.

We have begun to study cells in primary tissue culture of rat hippocampus, in order to investigate the properties of GABAergic interneurons in more detail than is possible in the hippocampal slice preparation. Hippocampi were dissected from fetal rats (17-19 days gestation), incubated with 0.1% trypsin and dissociated by trituration. The tissue was suspended in MEM + 10% fetal calf serum and plated out on polylysine coated Falcon 35 mm dishes. Cultures were maintained in 10% CO_2 at 37°C and subsequently fed weekly with an astrocyte conditioned medium of defined composition (N2). Rabbit antibody to human glial fibrillary acidic protein (GFA) and a mouse monoclonal antibody to a purported neuron specific marker, A_2B_5, were used to provide an initial characterization of cell types present. Using an indirect fluorescence technique, cells were shown to bind either anti-GFA or anti-A_2B_5, but not both. Many cells did not bind either antibody and although a few of these were flat fibroblast-like cells, most were process-bearing much like the A_2B_5 population. These cells may be oligo - droglia or undifferentiated neurons or astrocytes. Two cells identified electrophysiologically as neurons were shown subsequently to bind anti-A_2B_5 but not anti-GFA. The gigohm seal patch clamp technique was used to study single channel outward currents activated by morphine (1-10 μM) in an intact cell configuration. The patch pipette contained recording medium plus drugs. Such single outward currents were not observed in the absence of morphine or, in one experiment, when 1 μM naloxone + 1 μM morphine was present. Both single channel amplitude and percent open time increased when pipette voltage was made positive (15-50 mV). The open time could be exceptionally long (> 400 ms) although interrupted by short closures. Opening rate declined slowly during prolonged hyperpolarizing steps. The evidence, still incomplete, is consistent with a slow, voltage dependent outward current having properties reminiscent of I_{K2} in cardiac muscle. In other experiments the ability of cultured hippocampal cells to take up labeled GABA was examined. Cells 1-3 weeks in culture were incubated at room temperature with 3H-GABA, in the presence or absence of 150 mM Na. A Na-dependent uptake of GABA was demonstrated that was linear for approximately 30 min. The initial rate of uptake was half-maximal at 2-15 μM GABA and was inhibited by cis-4-OH-nipecotic acid (1-100 μM) and THPO (10-1000 μM), which are blockers of GABA uptake in other systems. In conclusion these primary cultures appear to be appropriate for the investigation of opiate receptor linked ion channels and GABAergic function. Supported by DA-02360, NS-16692 and the United Way Medical Research Fund.

328.9 DIRECT MEMBRANE ACTIONS OF DYNORPHIN ON PYRAMIDAL CELLS IN RAT HIPPOCAMPAL SLICES. M.F. Pacheco, J.M. Walker*+ and H.C. Moises. Dept. of Physiology and Mental Health Research Institute+, University of Michigan, Ann Arbor, MI 48109.

Our previous in vivo electrophysiological studies in rat (Walker et al.,Science 218,1982) suggested a potent "non-opiate" inhibitory action of Dynorphin 1-17 (DYN) on the activity of CA1 and CA3 hippocampal pyramidal cells (HPCs). In this study, extra- and intracellular recording techniques were used to further examine the actions of DYN in hippocampus. Rat hippocampal slices (500μm thick) were prepared using conventional techniques and maintained at 32°C either under static bath conditions or under superfusion (0.05ml/min) of normal media. Electrodes for intracellular recording (90-150 MΩ) were filled with 4M KAc (pH= 7.3). DYN (4μM, in normal media) was applied directly onto the slices by pressure ejection from a second micropipet (tip diameter 3-6μm)

Extracellular recordings from spontaneously active CA1 and CA3 HPC revealed two distinct populations of neurons with different firing patterns. One showed bursting activity (4-9 spikes/burst) and a discharge frequency of 1-3 bursts/sec, while the other fired single spike discharges at rates of 3-14 spikes/sec. Local application (100msec, 10 psi) of DYN had a predominantly inhibitory action on the spontaneous activity on both neuronal populations. However, this action differed considerably between the two groups. On neurons having single spike firing activities, DYN elicited an initial excitation (lasting 2-6 sec) followed by a gradual inhibitory response of prolonged duration (more than 15 - sec). This inhibitory phase was due more to a decrease in action potential (AP) amplitude than to a decrease in the firing frequency per se. In neurons presenting bursting firing patterns, DYN produced a rapid decrease in the discharge frequency accompanied by gradual reduction in spike amplitude, without reduction in the number of spikes per burst.

Local application of DYN (100msec, 10 psi) produced rapid (2-7 sec onset) changes in the membrane potential (Em) of CA1 neurons. Maximal effects were observed within 7 sec and recovery obtained within 20 sec - 2 min. The magnitude and direction of these effects appeared to be dependent on the initial Em. Whereas depolarizations were produced at an Em more negative than -56 mV, the effects of DYN changed to hyperpolarization at a less negative Em. The onset of the depolarizing action of DYN was often associated with the generation of APs. On the other hand, a decrease in the amplitude of spontaneous APs was observed during DYN-induced hyperpolarization.In conclusion, these results indicate that DYN has direct membrane actions on HPC which may account in part for our previous electrophysiological findings obtained with extracellular recording. (Supported by IRFP 1 FO5 TW03215-01 to M.P. and a Rackham Faculty Research Grant to H.M.)

328.10 DIFFERENTIAL RESPONSES OF HIPPOCAMPAL NEURONS TO ENDOGENOUS OPIOID PEPTIDES, AND OPIATE ALKALOIDS, SUGGEST MULTIPLE OPIATE RECEPTORS. S.J. Henriksen, G. Chouvet*, and F. Bloom. A.V. Davis Center, The Salk Institute, San Diego, California 92138 and *INSERM U171, University Claude Bernard, Lyon, France.

Previous neuropharmacological studies investigating responses of rat hippocampal neurons to exogenously applied opioid peptides (and opiate alkaloids) have demonstrated excitatory responses of these neurons to these substances. The experimental evidence, obtained from numerous investigations, suggests that the mechanism of these opiate/opioid elicited excitatory effects is one of disinhibition. Both in vivo electrophoretic studies, as well as studies utilizing the in vitro hippocampal slice preparation, have consistently demonstrated these disinhibitory effects, particularly in the CA1 cellular fields. Recent immunohistochemical studies in our laboratory (McGinty et al., PNAS 80, 589, 1983) have suggested the existence of the opioid peptide dynorphin A (1-17) in a major intrinsic hippocampal fiber system, the "mossy fiber" pathway. This system provides a major direct input to hippocampal CA3 pyramidal cells from the granule cells of the dentate gyrus. We have compared the effects of electrophoretically and micropneumatically applied dynorphin A(1-17), dynorphin A(1-8), and alpha-neoendorphin on hippocampal neurons to the effects of similarly applied opiate alkaloids having differential selectivity for the previously identified mu, kappa and delta opiate receptor subtypes. Studies were performed on Sprague-Dawley rats(N=120) lightly anesthesized with a Halothane-air mixture. As has been previously observed, mu and delta ligands (morphine, normorphine, and both enkephalin pentapeptides), produced over 90% excitatory effects on identified CA3 neurons (N=40). On the other hand, more selective kappa agonists (the dynorphin series) produced mixed excitatory (77%) or inhibitory (9%) effects. Ethylketocyclazocine also produced mixed responses (28% excitatory, 18% inhibitory). In addition, the dynorphin peptides and ethylketocyclazocine also produced a response pattern consisting of an initial inhibitory effect followed by a prolonged excitatory phase (dynorphin 15%, ethylketocyclazocine 56%). The most kappa selective agonist employed, U-50,488H, elicited the highest percentage of inhibitory effects (56%), and the lowest percentage of excitations (11%) observed. These results suggest that there may exist more than one opiate receptor sub-type in the rat hippocampus and that the excitatory or inhibitory effects observed on CA3 neurons may be due to specific functional roles of different endogenous opioid peptides and their precise hippocampal anatomical distribution. (Supported by DA 01785 and the Klingenstein Foundation.)

328.11 RESPONSE OF GLOBUS PALLIDUS NEURONS IN RAT TO MICROIONTOPHORETICALLY APPLIED MORPHINE, MORPHICEPTIN, DYNORPHIN AND MRZ 2549. R. D. Huffman and J. M. Frey. Division of Neuropharmacology, Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78284

While the globus pallidus of the rat brain has been shown to have only moderate to diffuse opiate receptor density, it has been shown to contain moderate to high concentrations of several opioid peptides (enkephalins, dynorphin). We have previously demonstrated that morphine and met-enkephalin have a predominatly depressant effect on the spontaneous discharge of pallidal neurons (Frey, J. M. and Huffman, R.D., Neurosci. Abst. 7:577, 1981). The present study was conducted in order to compare the effects of the microiontophoretic application of mu opiate receptor agonists (morphine and morphiceptin) and kappa opiate receptor agonists (dynorphin and MRZ 2549) on spontaneous and/or glutamate-evoked single unit activity within globus pallidus. Male Sprague-Dawley rats weighing 190-250g were anesthetized by an intraperitoneal injection of chloralose-urethane (25 mg/400 mg) and a left craniectomy was performed to expose the cortex overlying the globus pallidus. Morphine HCl (0.1M, pH 4.8), morphiceptin (0.1M in 100 mM NaCl), dynorphin 1-13 (10 mM in saline), MRZ 2549, a proposed kappa agonist (courtesy of Dr. Merz, Boehringer Ingelheim) (10 mM in saline), naloxone (0.1 M, pH 4-5) and L-glutamate (0.5 M, pH 8.0) were applied by microiontophoresis from 7 barrel glass micropipettes. Changes in spontaneous and/or glutamate-evoked discharge of single globus pallidus neurons were monitored before, during and after application of these drugs (alone or in combination with naloxone). The response of pallidal neurons to these different opiate agonists and naloxone are summarized below:

	Inhibit	Excite	No Effect	Naloxone Antag
Morphine	54%(18)	18%(6)	27%(9)	29%(4/14)
Morphiceptin	50%(3)	33%(2)	17%(1)	25%(1/4)
Dynorphin	42%(15)	17%(6)	42%(15)	82%(9/11)
MRZ 2549	89%(32)	0	11%(4)	42%(8/19)

Depression of neuronal discharge was the predominate effect elicited by all four opiate agonists; but a small percentage of neurons were excited by morphine, morphiceptin and dynorphin. The kappa agonist MRZ 2549 produced a powerful and consistent depression of neuronal discharge of most pallidal neurons to which it was applied. The excitatory responses of these drugs were much more readily antagonized by naloxone than the depressant responses. The responses to dynorphin were much more consistently antagonized by naloxone (82%) than were the responses to morphine (29%).

328.12 THE OPIATE QUASI-WITHDRAWAL SYNDROME IN RHESUS MONKEYS: COMPARISON OF NALOXONE-PRECIPITATED WITHDRAWAL TO EFFECTS OF CHOLINERGIC AGENTS. J.L. Katz* and R.J. Valentino (SPON: J.E. Barrett). NIDA Addiction Research Center, Baltimore, MD 21224 and The Salk Institute, San Diego, CA 92138.

The opiate quasi-withdrawal syndrome is a constellation of effects produced by acute administration of a drug to non-dependent subjects that resembles the withdrawal reaction following cessation of opiate treatment in dependent subjects. Two benzazocines (3-cyclopropylmethyl-1,2,3,4,5,6-hexahydro-8-hydroxy-6-methyl-benzazocine hydrochloride (UM-1046) and its 3-allyl analogue) have been reported to produce a quasi-withdrawal reaction in normal rhesus monkeys. These drugs are of interest because they are structurally related to the opiate mixed agonist-antagonists, cyclazocine and SKF-10047, respectively.

The present study was a dose-response comparison of effects of naloxone in morphine-dependent rhesus monkeys and effects of UM-1046 in normal subjects. Since the benzazocines likely produce their effects by release of acetylcholine, physostigmine was also studied in normal rhesus monkeys.

Rhesus monkeys maintained on regular morphine injections (3 mg/kg/6 hr) were treated with s.c. cumulative doses of naloxone two hours after a morphine injection. Doses of naloxone were administered at 30-minute intervals. Effects of UM-1046 and physostigmine were studied in non-dependent subjects in a similar fashion.

Reliable signs observed after naloxone (0.001 to 0.1 mg/kg) were grimacing, salivation, pacing, holding abdomen, retching, erection, rigid abdominal muscles, uncooperativity during handling, monkey lying on its side, dysphoric facies, miosis, fighting, dyspnea, tremor, hind-limb rigidity, and assumption of unusual positions. The frequency of these signs was dose dependent. Both UM-1046 (1.0 to 10.0 mg/kg) and physostigmine (0.1 to 0.56 mg/kg) produced some of these signs in normal monkeys, however, absent after either cholinergic agent were the following signs: grimacing, pacing, fighting, miosis, and rigid abdominal muscles. Additionally, the cholinergic drugs produced ataxia which was not observed after administration of naloxone to dependent monkeys.

While the cholinergic compounds produced many of the signs of precipitated withdrawal, the constellation of effects was not identical to that produced by naloxone. (Supported by USPHS Grant DA-00254.)

328.13 CENTRIFUGAL ANTICONVULSANT SYSTEMS & OPIATE-MEDIATED CATALEPSY. L.R. Watkins[1], H. Frenk[2] and D.J. Mayer[1]. Dept. of Physiology & Biophysics[1], Med. Col. Va., Richmond, VA 23298; Dept. Psychology[2], Tel-Aviv U., Ramat Aviv, Israel.

Opiates have long been known to produce proconvulsant, anticonvulsant & cataleptic effects. Our initial studies of intrathecal (IT) & cortical administration of opiates (Frenk et al., this vol) revealed 2 intriguing phenomena: (1) seizure activity following IT opiates was potentiated in spinalized rats, suggesting that descending spinal pathways inhibit convulsant activity in the cord; & (2) IT administration of methadone & DALA, but not morphine, produced catalepsy. The present experiments were aimed at: (1) defining the spinal pathways mediating anticonvulsant effects on IT morphine & (2) comparing the cataleptic & electrographic effects of various doses of IT & cortically applied opiates.

In rats, IT morphine (400 µg) applied to the lumbosacral cord elicits convulsive activity in the hindlimbs followed by repetitive myoclonic twitches (Frenk et al., this vol). Systematic comparisons of various spinal lesions revealed that (1) bilateral dorsolateral funiculus lesions potentiate seizure frequency & duration while not increasing twitch frequency, (2) large ventral cord lesions potentiate myoclonic twitch frequency while not increasing seizure activity, & (3) dorsal column, corticospinal, spinal gray, &/or ventromedial lesions did not potentiate convulsant activity.

Regarding cataleptic effects, IT DALA (34.35,137.5, 500 µg) & methadone (400 µg) induced catalepsy that was reliably reduced by systemic naltrexone (25 mg/kg). Equimolar IT morphine did not produce comparable catalepsy, but rather convulsive behavior. However, at 100 & 400 µg morphine, animals remained in bizarre postures upon culmination of convulsive behavior, only moving upon onset of renewed convulsive activity. These apparent cataleptic effects, but not the convulsive behavior, were antagonized by naltrexone.

When the same doses of DALA and methadone were microinjected onto the cerebral cortex of rats, both compounds induced depression of cortical EEG, resembling spreading depression. DALA induced depression lasted longer than that induced by equimolar methadone, following the same rank order of potencies as in inducing spinal catalepsy. Morphine, at doses equimolar with methadone & DALA, did not induce EEG depression, but rather convulsive seizures.

In view of the observations that DALA & methadone (1) induced similar electrographic and behavioral effects following cortical & IT administration, as opposed to morphine & (2) have the same rank order of potency in inducing spinal catalepsy & cortical depression we suggest that the physiological mechanisms underlying these two effects may be the same. Grant DA 00576.

328.14 BEHAVIORAL AND ELECTROGRAPHIC EFFECTS INDUCED BY INTRATHECALLY AND CORTICALLY APPLIED MORPHINE ARE DIFFERENT FROM THOSE INDUCED BY D-ALA^2-METHIONINE-ENKEPHALIN-AMIDE. H. Frenk[1], L.R. Watkins[2] and D.J. Mayer[3]. Dept. Psychology[1] Tel-Aviv University, Ramat Aviv, Israel; Dept. Physiology & Biophysics[2], MCV, Richmond, VA 23298.

The systemic administration of high doses of morphine causes repeated electrographic and behavioral seizures in the rat. These convulsions occur undiminished in morphine-tolerant animals and are not prevented, but rather potentiated, by naltrexone. In marked contrast to these observations stand the consistent reports that the electrographic seizure activity induced by intracerebroventricular (ICV) morphine and endogenous opioids is not accompanied by convulsions, shows tolerance and cross-tolerance to morphine, and can be blocked by opiate antagonists. Morphine, injected ICV, is known to reach different areas in the brain than when injected systematically. We therefore hypothesized that the nonspecific convulsant action of systemic morphine is mediated by areas not in the immediate proximity of the ventricular system, in particular the cerebral cortex and spinal cord.

Intrathecal morphine (25-400 µg) microinjected onto the lumbosacral cord elicits convulsive activity in the hindlimbs of rats, which is followed by myoclonic twitches lasting for about one hour. Supraspinal structures are not involved in this activity, as high thoracic spinalization does not eliminate, but rather potentiates this activity (see Watkins et al., this vol). This potentiation consists both of increased twitch frequency, and of prolonged duration of twitches (over 2 hours). When a similar range of morphine doses are microinjected onto the cortex, electrographic spikes and convulsive (at 400 µg) Stage 1-4 seizures ensue. The effect of neither intrathecally nor cortically applied morphine is abolished either by systemic pretreatment with naltrexone (25 mg/kg) or by prolonged preexposure to increasingly higher doses of systemic morphine. D-ala^2-methionine-enkephalinamide (DALA, 34.35,137.5 and 550 µg) in marked contrast to the results obtained with morphine, induces depression in cortical EEG when applied to the cortex, and prominent hindbody catalepsy when injected intrathecally at doses equimolar to morphine (see Watkins et al., this vol), also suggesting that morphine's convulsant action is not mediated by specific opiate receptors.

It is concluded that the spinal cord and cerebral cortex are anatomical substrates for the nonspecific convulsive action of systemically administered morphine. Grant DA 00576.

328.15 THE EFFECT OF THE DAY-NIGHT CYCLE ON MORPHINE INDUCED CONVULSIONS IN KINDLED MICE. A. Mansour and E.S. Valenstein. Dept. Psych. & Neurosci. Lab., Univ. Mich., Ann Arbor, MI, 48109.

Numerous studies in the literature have indicated that the effects of morphine vary in relation to the day-night cycle. For instance, Frederickson, Burgis, and Edwards (Science, 1977, 198, 756-8) report that animals show a peak sensitivity to morphine analgesia during the dark phase of the day-night cycle. Ayhan (Comm. J. Pharm. Pharmac., 1974, 26, 76-8) has found similar results for morphine induced locomotion. These psychopharmacological reports are complemented by Naber et al (Neuroscience Letters, 1981, 21, 45-50) findings demonstrating that the number of opiate receptors also show circadian fluctuations, with a peak during the dark phase. As part of a larger study examining changes in opiate sensitivity following repeated convulsions, we examined the effect of diurnal rhythm on the incidence of morphine induced convulsions in kindled mice. Nineteen C57BL/6J mice were implanted with chronic bipolar electrodes aimed at the amygdala (coor. B-1.5, 3.5 lat., 4.5mm from skull). The electrodes consisted of two twisted teflon coated stainless steel wires (0.006 inch diam.) soldered to microminiature pins. The animals were maintained on a 12 hr on/12 hr off light-dark cycle with the lights going on at 0500 hrs. All the mice received daily stimulation (1 sec, 60 Hz, 50μAmp) between 0900 and 1200 hrs until they reached a criterion of 7 consecutive generalized convulsions (Stage 5). Three days following their last convulsion the animals were divided into 3 groups. Two groups received either 30mg/kg (N=7) or 60mg/kg (N=6) morphine sulfate (i.p.) between 0800-1100 hrs. The third group of animals (N=6) was injected with 30mg/kg morphine sulfate between 1800-2000 hrs. Following their morphine injection, the animals were observed for 90 minutes and the incidence of convulsions was recorded.

Our results indicate that the responses of kindled animals to morphine vary with day-night cycle. Of kindled animals given 30 mg/kg morphine during the dark phase 83% showed clonic convulsions whereas only 29% of the animals given morphine during the light phase showed convulsions (30mg/kg) (Chi sq.=3.9, df=1, $p<.05$). In addition, only 83% of the animals treated with 60mg/kg morphine during the light phase convulsed again suggesting a shift to the right in morphine sensitivity during the light phase. These changes in susceptibility to morphine convulsions with day-night cycle are consistent with the variations observed by other investigators in morphine induced locomotion and analgesia and are correlated with the diurnal fluctuations observed in opiate receptors. These data are also consistent with findings that animals are more susceptible to audiogenic seizure (Halberg et al, Science, 1958, 128, 657-8) and kindled convulsions (Freeman, Behav. Neural. Biol., 1980, 30, 231-5) during the dark phase of their diurnal cycle.

328.16 INTRACEREBRAL OPIOIDS AND MORPHINE: KINDLING OF SEIZURES AND HANDLING-INDUCED POTENTIATION OF EPILEPTIC EFFECTS. D.P. CAIN and M.E. Corcoran. Depts. of Psychology, U. of Western Ontario,London, Ontario N6A 5C2 and U. of Victoria, Victoria, B.C. V8W 2Y2 CANADA.

Intracranial administration of morphine (MOR), met-enkephalin (M-ENK), or β-endorphin (β-END) can produce pathological changes in brain activity, including epileptiform spiking. However, little or no convulsive behavior has been reported to result from administration of these substances. We have used the kindling technique to repeatedly infuse small amounts of opiate or opioid into the anterior (ant) or basolateral (bl) amygdala (amyg) or the ventral hippocampus (v hpc). Drugs were infused in a volume of 1 μl or less at 0.029 μl/sec at 48-hr intervals. Infusion of 10 μg MOR into the ant amyg induced strong epileptiform spiking in half of the rats so treated. Repetition of the infusions led to tolerance after a mean of 3.2 infusions, and in no rat was there evidence of a kindling-like increase in the epileptiform response. Infusion of MOR into the bl amyg failed to produce any epileptiform effects. Infusion of 10 μg M-ENK or β-END into the bl amyg and of β-END into the v hpc induced long trains of high-amplitude and frequency epileptiform spikes. Repetition of the infusions resulted in the gradual development of bilaterally generalized convulsions after a mean of 3.8 infusions of M-ENK and 2.0 infusions of β-END.An equal number of infusions of saline failed to cause any epileptiform effects in control rats, and the infusion of opioids into the ant amyg was without effect. Smaller doses of the opioids induced weaker dose-related EEG and convulsive responses. Naloxone (10 mg/kg ip) blocked or terminated spiking and convulsions in opiate- or opioid-treated rats, suggesting that the effects of these drugs were mediated by an interaction with opiate receptors. These results support the view that endogenous opioids might play a role in convulsive seizures.

An unexpected result was the potentiation by handling and conspecific threat of seizures evoked by infusions of β-END into the bl amyg or v hpc, and of MOR into the ant amyg. Strong bursts of high-frequency spikes were observed in some animals during and after handling and threat, and in some cases the EEG and convulsive responses were markedly stronger than those that had occurred when the animals were undisturbed. No epileptiform or convulsive phenomena were observed when these animals were handled after control infusions of saline. Acute stress promotes the secretion of opioid peptides into the brain and circulation, and even gentle handling can have stressful consequences in the rat.It is possible that handling increases the release of opioid peptides in specific brain regions, and that these then interact or summate with infused MOR or β-END to potentiate their epileptiform effects.

Supported by NSERC (D.P.C.) and MRC and BCHCRF (M.E.C.).

PEPTIDES: BIOSYNTHESIS AND METABOLISM II

329.1 SUBTANCE P TURNOVER: REGIONAL DIFFERENCES AND PHARMACOLOGICAL RESPONSIVENESS. M.J. Bannon and M. Goedert*. MRC Neurochemical Pharmacology Unit, MRC Centre, Medical School, Hills Road, Cambridge CB2 2QH, U.K.

The concentration of neuropeptides in the central nervous system shows marked regional differences. However, for other neurotransmitters it has been demonstrated that the measurement of absolute levels does not represent a good index of physiological activity (1). An indication of peptide turnover is thus required in order to understand the role of neuropeptides in brain function. Since it is not yet possible to inhibit specifically the enzymes involved in neuropeptide biosynthesis or degradation we measured the rate of utilization of the neuropeptide substance P (SP) after inhibition of rat brain total protein synthesis. A dose of 900 μg/kg of cycloheximide (s.c., 4 h prior to ^{3}H amino acid administration) inhibited by 85% the incorporation of ^{3}H amino acid into brain proteins; up to 200 fold higher doses resulted in no greater inhibition of protein synthesis. Four hours after the low dose of cycloheximide, SP concentrations (determined by radioimmunoassay) in the substantia nigra, hypothalamus, striatum, frontal cortex and nucleus accumbens were unchanged. Previous investigations have shown that dopaminergic drugs can influence SP levels in various brain areas (2,3). This has provided indirect evidence for an interaction between SP and dopamine systems. In the present study, the injection of the dopamine receptor antagonist haloperidol (1 mg/kg, i.p. 2 h before sacrifice) elicited increases in SP in the striatum and frontal cortex, but no change was measured in substantia nigra, hypothalamus and nucleus accumbens. Nevertheless, in all of these brain regions, haloperidol injection 2 h after cycloheximide (and 2 h before sacrifice) caused a dramatic cycloheximide-mediated depletion of SP of the order of 30-60% In the ventral tegmental area, either 4 h cycloheximide pretreatment or 2 h haloperidol pretreatment caused approximately a 50% decrease in SP, while the combination elicited no further depletion. In conclusion, changes in the rate of decline of SP following protein synthesis inhibition may provide an index of turnover useful for a further characterisation of the physiology and pharmacology of SP. This method may be of general applicability to the study of neuropeptides.

References

1. Cooper, J.R., Bloom, F.E. and Roth, R.H. The biochemical Basis of Neuropharmacology. Oxford University Press, 1982.
2. Hanson, G. et al. Neuropharmacol. 20: 541-548, 1981.
3. Hong, J.S. et al. Neuropharmacol. 17: 83-85, 1978.

329.2 IN VITRO SYNTHESIS AND TRANSPORT OF SUBSTANCE P IN EXPLANTS OF GUINEA PIG NODOSE GANGLION-VAGUS NERVE. DB MacLean,* S Lewis,* and W Dodge*(Spon: M Tytell). Department of Medicine, Bowman Gray School of Medicine, Winston-Salem, NC 27103

We are using the transport model to study factors regulating the synthesis of substance P within the vagus nerve. In preliminary studies, we determined the bulk transport of SP in the guinea pig (GP) cervical vagus nerve and the contribution to that transport by the nodose ganglion (NG). 24h following ligation of the vagus 2 cm distal to the NG, SP immunoreactivity proximal to ligation was 2217±393 pg (mean ± SD, n=6). Net 24h transport was reduced to 1131±242 pg ($p<.01$) following simultaneous supranodose crush and distal ligation performed in separate animals.

For in vitro studies, GP's were anesthesized, perfused with iced Ringer's, and nodose ganglia and 2-2.5 cm of adjacent nerve resected. Nerves were suspended on 0.3% agar, M-199, 10% FCS and incubated in 95% O_2/5% CO_2. Prior to explantation, the vagus was ligated 2.0 cm distal to the ganglion. The content of SP (pg, mean ± SD, n=4-6) in the nodose ganglion, 3 mm segments of vagus nerve, and 3 mm proximal to ligation were as follows:

Hours	Nodose	Vagus (3mm)	Prox. Seg (3mm)
0	353±94	113±13	109±56
8	282±51	97±22	564±119
24	471±179	128±35	795±161

These results suggested in vitro synthesis corresponding closely to the in vivo results. To confirm synthesis, in separate experiments, similar explants were incubated with 35-S methionine (2 nerves, 200 uCi/dish). Nerve segments, acid and C-18 (Sep-pak) extracted, were subjected to two consecutive HPLC separations using different solvent systems. Recovery was monitored, and radioactivity peak identity determined, using UV detection of added SP carrier. The second HPLC purification resulted in a single peak of radioactivity co-eluting with SP carrier. Net radioactivity in this peak, following oxidation with H_2O_2, co-eluted with SP-sulphoxide using a 3rd solvent system.[2] ^{35}S incorporation into proximal segment SP was 552 DPM (pool of 4 nerves) at 16h, and reduced to 290 DPM at 16h following chase with cold media at 8h. At 24h, no further increase was noted. Radiolabeled SP in NG at 24h was 238 DPM (4 pooled ganglia).

Our results confirm in vitro synthesis of SP within NG-vagus nerve explants. 35-S incorporation studies correlate well with in vivo and in vitro evidence of de novo synthesis as determined by RIA, and support the validity of the transport model for studying SP synthesis in this important nerve.

329.3 IN VIVO BIOSYNTHESIS OF OXYTOCIN, VASOPRESSIN AND NEUROPHYSIN AND THEIR TRANSPORT TO PITUITARY, BRAIN STEM AND SPINAL CORD IN INDIVIDUAL RATS. J.D. White, J.E. Krause and J.F. McKelvy. Dept. of Neurobiology & Behavior, SUNY at Stony Brook, Stony Brook, NY 11794

There is a growing body of literature that supports the notion that oxytocin (OXY) and arginine-vasopressin (AVP), in addition to their roles as endocrine hormones, may serve as neurotransmitters or neuromodulators within the central nervous system (CNS). Previous anatomical evidence suggested that cells in the paraventricular nucleus of the hypothalamus (PVN) project to the brain stem, specifically the nucleus of the solitary tract (NTS) and to the sympathetic preganglionic cells in the intermediolateral cell column of the spinal cord. As these latter areas are known to be involved in the regulation of autonomic nervous system function, it is important to chemically identify putative transmitters in these systems so that we may better understand the physiological regulation of these systems. We describe here methods for studying the in vivo biosynthesis and transport to endocrine and descending sites of OXY, AVP and their neurophysins (Np) in individual rats.

Rats were bilaterally cannulated in the PVN (A:0.2 mm, V:7.3 mm, L:0.5 mm with respect to bregma). Twenty-four hours later a 2 hr. pulse of ^{35}S-cysteine (250 µCi/cannula) was infused into the rat using an Alzet osmotic mini-pump delivery system at a flow rate of 1 µl/hr. After a 10 hr. chase period, during which time artificial ECF was infused through the cannulas, the rats were sacrificed by decapitation. The brain was rapidly removed and frozen while the neural lobe and spinal cord was removed from the animal. The spinal cord was dissected into segments T_1-T_4 and T_{12}-L_2 and the PVN and NTS were punched from frozen coronal brain sections. All tissues were homogenized in 2 N acetic acid and carrier OXY, AVP and somatostatin peptides were added.

^{35}S-cysteine-labeled OXY,AVP and Np were purified by adsorption and elution from octadecyl-silica cartridges, linear HPLC gradient analysis, chemical modification, exponential gradient HPLC analysis followed by linear HPLC gradient analysis using an ion-pairing buffer system.

Using this approach, we have identified ^{35}S-labeled material which co-purifies with OXY and AVP from the PVN, neural lobe and NTS. ^{35}S-labeled Np has been identified in these extracts by HPLC co-migration, immunoprecipitation and tryptic mapping. Preliminary studies have also demonstrated ^{35}S-labeled OXY in the spinal cord extracts. (NSF BNS-7684506)

329.4 HPLC ANALYSIS OF ARGININE-VASOPRESSIN BIOTRANSFORMATION BY HIPPOCAMPAL RAT MEMBRANES. M.G. Costantini* and A.F. Pearlmutter* (SPON: G.F. Ayala). Dept. of Biochemistry, Medical College of Ohio, Toledo, OH 43699.

We have demonstrated previously that [^{3}H-Tyr]arginine-vasopressin (AVP) binds specifically to synaptic (12K) and microsomal (100K) membranes from different areas of rat brain. The binding was saturable and required the presence of 10mM $NiCl_2$. Bound AVP was dissociated by 10mM EDTA within 15-20 min which indicated that Ni is involved in the binding reaction.

Because we have shown previously that AVP preferentially binds to the synaptosomes in the hippocampus, this region was chosen for more in depth examination. We have analyzed and compared the metabolism of [^{3}H-Tyr]AVP and [^{3}H-Phe]AVP after incubation for 90 min with 12K or 100K hippocampal membranes. For analysis by HPLC, acetonitrile (AN, final 50%) was added to the sample. After centrifugation, the supernatants were chromatographed through a C_{18} reversed phase column using a linear gradient of AN and 50mM ammonium acetate, pH 4.1, (12-32%), and the radioactivity in each fraction was determined.

Analysis of the 100K samples incubated with [^{3}H-Tyr] or [^{3}H-Phe]AVP in the presence of 10mM $NiCl_2$ at pH 7.8 shows one main peak of radioactivity which coelutes with nonradioactive AVP and represents 65% of the total radioactivity, a more polar peak (peak II) which represents 7-12% of the total, and a very polar peak (peak I) in the void volume which represents 2-5% of the total and coelutes with tyrosine or phenylalanine. Superimposable profiles are obtained with 12K incubates. When 10mM EDTA is added to 12K incubates after 90 min, the amount of radioactivity in the AVP peak increases by approximately 9% while peaks I and II remain unchanged.

Incubation in the presence of EDTA for 90 min or in the absence of $NiCl_2$ changes the radioactive HPLC profiles dramatically. The amount of radioactivity in the AVP peak drops by 40% for 12K incubates and by 70% for 100K incubates, the amount in peak II remains essentially unchanged, while that in peak I (amino acids) increases proportionally.

In conclusion, our results indicate that: a) vasopressin is quickly degraded by hippocampal membranes in the absence of Ni or in the presence of a chelating agent, b) another role of Ni is that of inhibiting peptidase activity, c) intact vasopressin is the actual ligand bound to the receptor sites. Our findings suggest an alternative mechanism of regulation of vasopressin action in the brain by which degradation of vasopressin would inhibit its action, while protection from cleavage (by Ni or other metal ions) would permit binding and the initiation of subsequent effects. This research was supported by NIH grant NS 17848.

329.5 PROTEOLYSIS OF CHOLECYSTOKININ OCTAPEPTIDE IN BRAIN MEMBRANES. L. Steardo,* M. Knight, C.A. Tamminga, T.N. Chase. NINCDS ETB, Bethesda, Maryland 20205; Maryland Psychiatric Research Center, U. of Maryland, Baltimore, Maryland 21228.

Cholecystokinin octapeptide (CCK8) is a presumed neurotransmitter in the central nervous system. Information detailing the metabolic pathway of synthesis and degradation of the peptide is critical in designing exogenous pharmacologic probes to manipulate the activity of the system. We describe the metabolic products and time course of the catabolism of CCK8 by CNS neural membranes. CCK8 (Boehringer Mannheim) was incubated at a concentration of 10^{-5}M with the P_2 fraction of rat neural membranes (485 ug protein/ml) at 37° buffered with 0.05M ammonium acetate pH 7.0. At specified time points (2.5, 5, 10, 20 min) aliquots were sampled from the incubation mixture; the reaction was terminated in boiling water. After centrifugation, the supernatant was lyophilized, dissolved in water, analyzed by HPLC on a C_{18} column using a mobile phase of 0.1% phosphoric acid and a gradient of acetonitrile at a 2 ml/min flow rate. Peaks were detected by UV absorbance at 280 nm using 0.02 absorbance units as full scale. Chromatographic peaks were collected, lyophilized, and hydrolyzed in 3N mercaptoethanesulfonic acid for 22 hr at 110°; the hydrolyzed peaks were submitted to amino acid analysis for composition determination. Simultaneously, peaks were identified by co-chromatography with available standards. The chromatographic separation of the timed CCK8 incubation mixtures resulted in a number of peaks related to CCK which increased linearly over time. The following peaks were identified in the 20 min sample: Asp-Phe-NH_2 (6 min); Trp and Asp-TyrOSO$_3$H-Met-Gly (9 min); Gly-Trp (12 min); Met-Asp-PheNH$_2$ (24 min); Trp-Met-Asp-PheNH$_2$ (24 min); Asp-TyrOSO$_3$H-Met-GlyOTyr-Met-Asp (27 min); Gly-Trp-Met-Asp-PheNH$_2$ (27 min); CCK8 (31 min). Two peaks obtained consistently at 26 and 28 min were considered oxidation products of CCK8. The 27 min peak (CCK5) increased then decreased over time while the 24 min peak (CCK4) showed a delayed then linear increase indicating a possible precursor-product relationship between these two peptides. The 25 min peak (CCK 26-32; CCK heptapeptide) showed a strong linear increase over time suggesting its production by the enzymatic action of first a deamidase then a carboxy-peptidase and its status as an intermediate product. We have found this heptapeptide fragment to bind to cortical CCK receptors; thus it may have physiologic activity in brain. Whether the heptapeptide is an in vivo product remains to be determined.

329.6 IN VIVO SYNTHESIS OF ^{35}S-CHOLECYSTOKININ IN THE RAT PIRIFORM CORTEX. K. Gysling* and M. C. Beinfeld* (SPON: M. J. Meldrum). Dept. of Pharmacol., St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

Goltermann et al. (J. Biol. Chem., 255:6181, 1980) have previously reported the in vivo synthesis of CCK in cerebral cortex after intraventricular injections of ^{35}S-methionine (^{35}S-met) using immunoadsorption and chromatography on Sephadex-650 XF columns. Our aim was to further study in vivo biosynthesis of CCK using direct stereotaxic injections into specific areas of the cerebral cortex and a more conclusive identification of biosynthetic products by immunoprecipitation and HPLC. For this purpose we selected the piriform cortex, an area containing high concentration of CCK (Beinfeld et al., Brain Res., 212:51, 1981) and numerous CCK cell bodies detected immunocytochemically (Hendry et al., PNAS, 80:2400, 1983).

Male, Sprague-Dawley rats (250-300 g) were injected with ^{35}S-met (100 µCi) in three sites in the piriform cortex. Rats were killed at different times after the injection and the cortical area was quickly removed and sonicated in 90% methanol. The methanol extract was dried and resuspended in buffer. Methanol extracts subjected to HPLC analysis showed several peaks of radioactivity. One of these peaks co-eluted with CCK8 standard and with the endogenous CCK8 as determined by radioimmunoassay. Part of the counts co-migrating in the peak with CCK8 were precipitated with a specific CCK antiserum. This immunoprecipitation was blocked with the addition of an excess of CCK8. Methanol extracts subjected to immunoprecipitation prior to HPLC yielded a radioactive peak for free ^{35}S-met and only one additional peak that co-eluted with standard and endogenous CCK. The recovery of this radioactive peak by immunoaffinity chromatography was blocked by an excess of CCK8. Using these methods newly synthetized ^{35}S-CCK was detected in the piriform cortex 1 hour after the injection of ^{35}S-met in the area. Maximum synthesis (2173±228 dmp/cortex sample) was observed after 2 hours, remaining high for at least 6 hours.

It is confirmed that ^{35}S-met injections in a discrete brain area can be used to study in vivo synthesis of CCK. The combined technique of immunoprecipitation with HPLC analysis used in this study convincingly demonstrated the formation of ^{35}S-CCK in the piriform cortex.

Supported by NIH Grant NS-18335 and a grant from the American Parkinson Disease Association.

329.7 THYROTROPIN-RELEASING HORMONE (TRH): DEGRADATION IN SERUM AND SPECIFIC CNS LOCI OF THE RAT. T.J. Cavanagh*, T.G. Hill*, W. Day*, R.W. Roeske*, J.M. Meyerhoff, and M.J. Kubek (SPON: J. Nurnberger). Depts. of Anatomy, Biochemistry and Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN 46223, and Dept. of Med. Neurosciences, Walter Reed Army Inst. of Res., Washington, D.C. 20012

Inactivation of TRH (pglu-his-proNH2) by serum and CNS enzymes may regulate its function as a releasing hormone and possible neurotransmitter/neuromodulator. However, its primary metabolic pathways have yet to be unequivocally determined. We have developed a two-dimensional TLC system to examine all known or suspected products of TRH degradation in serum and brain.

Serum or 170,000XG supernatant (S170) and particulate (P170) fractions of CNS sonicates (amygdala, AY; pyriform, PFM; striatum; ventral striatum; hippocampus; hypothalamus, HY) were incubated with [3H-glu] TRH (44.1 Ci/mmol) or [3H-pro] TRH (103 Ci/mmol) for 30 min at 37°C in 0.01M PO4, 0.15M NaCl, pH 7.5 (PBS). Peptide markers and [3H-glu] TRH were synthesized by liquid-phase techniques. Reaction products were extracted with MeOH, chromatographed with appropriate markers in solvent I, air dried, and developed in solvent II at 90°. Metabolites were stained, extracted with MeOH and quantitated by liquid scintillation.

Serum pglu-aminopeptidase (PAP) cleaved TRH rapidly to pglu (not degraded further) and his-proNH2, which either cyclized to cyclo-his-pro, or was degraded further to his-pro, pro and proNH2. The products at 30 min were: p-glu > pro > cyclo his-pro and his-proNH2 > his-pro > proNH2. No TRH-OH was formed.

All brain regions contained PAP and amidase. Most PAP (60-86%) was present in the P170, however, some pro, his-pro, and proNH2 were produced by both fractions. In contrast, TRH-amidase activity was cytosolic (90-98% in S170) except for HY. The TRH-OH formed in cytosol was not degraded further. PFM and AY exhibited the highest TRH-degrading activity of the six regions studied.

These results indicate that in serum, TRH is cleaved by PAP (which is rate limiting) to yield p-glu + his-proNH2. His-proNH2 is degraded by imidopeptidase, endopeptidase and amidase to produce pro and his as major products. In brain, PAP, imidopeptidase and amidase are localized in particulate and cytosolic fractions, whereas TRH-amidase activity is cytosolic. The differential distribution of enzymes degrading TRH may reflect variable activities of TRH or its metabolites in specific brain regions. This is supported by observations that PFM and AY contain high TRH degrading activity; have among the highest concentration of CNS TRH receptors (J. Neurochem. 38: 1649, 1982); contain appreciable amounts of TRH; and undergo the greatest change in TRH content following electroconvulsive seizures (Soc. Neurosci. Abstr. 8: 982, 1982). AM-28260 (MJK).

329.8 NEUROPEPTIDE SYNTHESIS AND METABOLISM IN VERTEBRATE RETINA D. Parkinson, and W.K. Stell. Department of Anatomy, U of Calgary Alberta, Canada.

Immunohistochemical methods have localized neuropeptides (e.g. enkephalins, sub. P.) to distinguishable subsets of amacrine neurons in the retinas of many species. The retina presents several useful advantages for examining the neurochemistry of neuropeptides: particularly attractive are, (a) the ease of technical manipulations without compromising the well being of the animal, (b) its level of activity can be controlled by modulation of its physiological stimulus. We have chosen to use S-35-methionine after intraocular injections, in vivo, since methionine is part of the amino acid sequence of Met-enkephalin (M-E), Substance P(SP) and vasoactive intestinal peptide (VIP).

Chickens were injected intravitreally with S-35-methionine (10-50μC, 1200/nmole) dissolved in saline. At various times the retinas were dissected out, homogenized in 1M acetic acid, boiled for 10 min. and then centrifuged. Peptides in the supernatant were resolved by hplc on a μBondapak C-18 column with acetonitrile (AN) gradients in aqueous trifluoroacetic acid. Authentic samples of M-E, Leu-enkephalin, SP and VIP could be readily resolved with > 95% recovery by these methods when added to retinal samples. Retinas obtained 3h. after injection contained substantial amounts of radioactivity which could be extracted by Sep-pak. Hplc of this fraction revealed several peaks of radioactivity, one of which appeared to coelute with M-E, at about 25% AN in the mobile phase. Rechromatography of this fraction on a Resolve-5μ C-18 column with phosphate buffer pH 4.5 and AN gradient resolved two peaks of radioactivity, one of which coeluted with M-E. Similar results were obtained 6h after injection except that less radioactivity appeared to co-chromatograph with M-E. This indicates retinal M-E is being metabolized with loss of radiolabel from the neuropeptide stores. Attempts to isolate S-35-labelled SP were confounded by the large amounts of radioactivity eluting around the expected retention time of this peptide.

In the rabbit, incorporation of S-35-methionine was examined after the intra-vitreal injection of 50 μC of label. Chromatographically identified M-E containing S-35 was found 2.5h post injection but by 5h the amount of radioactivity was considerably reduced. This indicates synthesis and turnover of M-E in rabbit retina also.

In conclusion, these results indicate that biosynthesis and turnover of M-E occurs in the retinas of several species. The factors which regulate these processes remain to be determined and the retina would seem to be an ideal tissue for such investigations.

Supported by Alberta Heritage Foundation for Medical Research.

329.9 CENTRAL METABOLISM OF ANGIOTENSINS. C.G. Camara, R.H. Abhold* and J.W. Harding. College of Veterinary Medicine, Washington State University, Pullman, WA 99164-6520.

The presence of central angiotensin receptors in the mammalian brain has been confirmed by several investigators using radioligand binding techniques. However, the interpretation of angiotensin binding data can be complicated by the rapid metabolism of angiotensins by brain membranes. In order to circumvent this problem researchers have utilized two complementary strategies: peptidase inhibitors have been included in the binding assay medium and binding parameters have been measured using angiotensin analogs that have lower degradation rates than the native angiotensins.

However, recent work in our laboratory indicates that peptidase inhibitors, while decreasing degradation of angiotensins, also decrease the binding of angiotensins to brain membranes. Additionally, we have used sensitive analytical techniques to demonstrate that in the absence of peptidase inhibitors most of the radioactivity displaced by cold angiotensin excess is not angiotensin but a metabolite of it.

These findings suggest that the commonly estimated values of the binding parameters of angiotensins to their putative receptors are, at best, composite values which reflect the binding both of angiotensins and of their metabolites to membrane sites.

329.10 RABBIT MUSCLE CARNOSINE SYNTHETASE: PURIFICATION AND GENERATION OF MOUSE ANTISERA. M. Grillo*, F. L. Margolis. Dept. Physiolog. Chem. & Pharmacol., Roche Institute of Molecular Biology, Roche Research Center, Nutley, N. J. 07110.

Carnosine synthetase is an enzyme responsible for the ATP dependent synthesis of several ω-amino-acyl histidine dipeptides. Among these are carnosine (β-Ala-L-His) and anserine (β-Ala-1methyl-L-His) found neural tissue and muscle and homocarnosine (GABA-L-His) found in brain and gut. β-Ala-L-Orn and β-Ala-L-Lys are also products of this enzyme. We have purified this enzyme nearly 3000-fold from rabbit muscle 100,000xg supernatants by ammonium sulfate precipitation followed by chromatography on hydroxyapatite and DEAE-cellulose. Electrophoresis of this material on non-denaturing polyacrylamide gels demonstrates the presence of several protein bands only one of which is associated with carnosine synthetase activity. The purified enzyme, which is quite unstable, has an apparent molecular weight of 500K, eluting from Ultrogel Ac 34 very close to ferritin. Carnosine synthetase exhibits Km values for histidine and β-alanine of about 200μM each with linear Lineweaver-Burk plots. In contrast, Lineweaver-Burk analysis of activity as a function of Mg^{++}/ATP concentrations gives non-linear plots. The significance of this observation in unclear but suggests Mg^{++}/ATP may also function as an allosteric modulator of this enzyme. In the course of studying this and evaluating various compounds as potential inhibitors of this enzyme, we have discovered that carnosine synthetase is inhibited by certain triphenylmethane and procion dyes at 10-100uM. This suggests these dyes may be useful as pseudo-affinity ligands for purification and studies of enzyme mechanism. Preliminary data indicate that these dyes may modify the interaction of Mg^{++}/ATP but not β-Ala or L-His with the enzyme.

Mice of four different strains have been immunized with partially purified enzyme and serum antibodies produced in two of these strains. The antibody-enzyme complex immobilized on a solid phase support retains almost full enzymatic activity. Cell fusions to generate monoclonal antibodies from these mice are underway. These antibodies should enable us to determine whether the carnosine synthetase activity in various tissues is due to the same enzyme and also permit cellular and subcellular localization of this enzyme by immunocytochemical techniques.

329.11 TRH INACTIVATION IN RAT BRAIN. P. Joseph-Bravo*, R. Garat*, J. Miranda*, G. Ponce* y J.L.Charli*. (SPON: B.Fuentes-Pardo). Centro de Investigación sobre Ingeniería Genética y Biotecnología, U.N.A.M., México.

Thyrotropin Releasing Hormone (TRH) is a neurotransmitter candidate in the Central Nervous System. For this, an inactivation mechanism must occur after it has interacted with its receptor. Degradation of TRH has been a subject of interest but also of considerable controversy. While the mode of its degradation has been characterized, been due to two identified enzymes: a pyroglutamilamino peptidase that produces a stable active metabolite (hispro-diketopiperazine) and a deamidase that gives rise to TRH-OH; the subcellular distribution of this enzimes is not at all clear and their physiological relevance unknown. Some reports find both of them in the soluble fraction of brain homogenates, while other groups claim that the pyroglutamilaminopeptidase is a particular enzyme. We have found this discrepancy to be due to the buffer in which the enzyme is being determined, and that indeed there is pyroglutamino peptidase activity in both fractions. The soluble enzyme is very sensitive (as previously reported by Bauer) to oxidation, requiring DTT and EDTA for its expression; the particulate on the other hand, resembles more to that reported for serum: it is inactivated by EDTA and does not degrade the artificial substrate: pyroglu NA. The presence of TRH degrading enzymes in membranes is of particular interest in searching an inactivating mechanism for this peptide once it is released into the synaptic cleft.

However, we equally investigated the possibility that TRH could be inactivated by an uptake phenomenon. We found that intact TRH is accumulated by hypothalamic slices by a time and energy dependent saturable phenomenon. The process is also inhibited by ouabain.

Work supported by CONACYT-PRONALSA and Instituto Mexicano de Psiquiatría.

329.12 INHIBITION OF OPIOMELANOTROPIN ACETYLTRANEFERASE BY [Pro^1, Nle^4]-α-MSH1-10 Amide. M.C. Chappell*, T.K. Sawyer*, C. Cummings* and T.L. O'Donohue (SPON: S.N. Pradahn). Experimental Therapeutics Branch and Neurosurgical Branch, NINCDS, NIH, Bethesda, MD 20205 and Upjohn, Kalamazoo, MI.

In previous studies, we have identified N^{α}-acetyltransferase activity in the neurointermediate (NI) lobe of the rat pituitary which acetylates α-melanocyte-stimulating hormone (α-MSH) and β-endorphin (Chappell, et al., Peptides 3:408, 1982). For the present study, we have designed and synthesized an α-MSH1-10 analog to inhibit this acetyltransferase.

The strategy underlying the design of the [Pro^1, Nle,^{4}]-α-MSH1-10 analog was as follows. As the terminal amino group of serine was apparently required for α-MSH acetylation to occur, blocking the terminal amino group might result in a peptide capable of binding to the acetyltransferase yet unable to be N-acetylated. To achieve this, proline which contains a secondary amine, was substituted for serine in the one position of the α-MSH1-10 analog. Norleucine was also substituted in the four position for methionine to prevent possible oxidation of the peptide. Only the first 10 out of 13 amino acids of α-MSH were included for the synthesized analog as the 1-10 region of α-MSH was known to contain the primary information necessary for enzymatic recognition and binding. Furthermore, elimination of the lysine on position 11 of the peptide prevented possible non-specific acetylation of the epsilon-amino group of the lysl residue. The C-terminal glycine was amidated to prevent exopeptidase degradation.

The [Pro^1, Nle^4]-α-MSH1-10 amide was used as a substrate in the enzymatic assay described previously (Chappell, et al., Peptides). A 12,000xg supernate fraction of rat NI lobe was incubated with 7.5 μM ^{3}H-Acetyl-Coenzyme A and 200 μM [Pro^1, Nle^4]-α-MSH1-10 amide at 37°C. The products were isolated by ion-exchange chromatography and reverse-phase HPLC and then counted. Incorporation of ^{3}H-acetate into the proline analog did not occur and indicated the analog was not a substrate for acetylation. Co-incubation of deacetylated α-MSH1-13 and the analog resulted in the blocking of ^{3}H-acetate incorporation into deacetylated α-MSH. The kinetic data revealed an apparent Km and Vmax for deacetylated α-MSH of 35 μM and 13 pmol/min/mg protein near a saturating level of ^{3}H-AcCoA. In the presence of the inhibitor, the apparent Km and Vmax for deacetylated α-MSH were 39 μM and 8.5 pmol/min/mg protein.

Surprisingly, the [Pro^1, Nle^4]-α-MSH1-10 amide did not inhibit acetylation in a competitive manner. The kinetic data indicated the analog to noncompetitively inhibit acetylation of deacetylated α-MSH. Studies are in progress to determine the mechanism of inhibition by this analog.

329.13 CHARACTERIZATION OF A PEPTIDE α-AMIDATION ACTIVITY FROM RAT HYPOTHALAMUS. R. EMESON* (Spon: R. Mains). Dept. of Neuroscience, Johns Hopkins University, Baltimore, MD 21205

A number of neuroendocrine peptides contain α-amide moieties at their carboxyl terminus. For many of these molecules, α-amidation is essential for optimal biological activity. Bradbury et al. [Nature 298:686-688 (1982)] identified a peptide α-amidation activity in porcine pituitary secretory granules that converts [^{125}I]-D-Tyr-Val-Gly to [^{125}I]-D-Tyr-Val-NH_2 plus glyoxylate.

Mono-[^{125}I]-D-Tyr-Val-Gly was used to identify α-amidation activity in a crude mitochondrial/synaptosomal pellet (P2) from rat hypothalamus. [^{125}I]-labeled substrate and product were separated by ion-exchange utilizing SP-Sephadex. Reversed-phase HPLC analysis verified the reaction product identification.

The pH dependence for the synaptosome-associated α-amidation activity was rather broad with an optimum near 8.0. A boiled enzyme blank showed no detectable activity (less than 0.1% conversion). The reaction was linear in both time and protein concentration. After repetitive freezing and thawing, 60% of the recovered α-amidation activity was soluble (3,000,000 g-min).

Based on the stimulatory effect of copper on pituitary α-amidation activity [Eipper et al. PNAS:in press], a variety of divalent cations were tested for their effect on hypothalamic α-amidation activity. Of those metals tested (Ca, Mn, Fe, Mg, Co, Cu, Ni, Sn, Zn, Cd), only copper was shown to have a stimulatory effect (17-fold), but optimal copper concentrations (~25μM) varied slightly between synaptosome preparations.

The conversion of a glycine-extended peptide into an α-amidated product plus glyoxylate could involve dehydrogenation and spontaneous hydrolysis (Bradbury et al.) or the action of a monooxygenase. Copper-containing metalloproteins often utilize cofactors: a variety of reduced and oxidized flavin, pyridine and pteridine nucleotides were tested. Of those factors tested, ascorbate appeared to have a maximal stimulatory effect (43-fold) at optimal concentrations (0.625mM). Catalase was necessary in the reaction mixture in order to observe the stimulatory effects of ascorbate on α-amidating activity, as seen for dopamine-β-hydroxylase. When the reaction was performed under a moist argon atmosphere, hypothalamic α-amidation activity was inhibited to 11% of control. Thus peptide α-amidation appears to be catalyzed by a copper-requiring monooxygenase that utilizes ascorbate.

Kinetic analyses have demonstrated Michaelis-Menten type kinetics for D-Tyr-Val-Gly as the varied substrate: the K_m for D-Tyr-Val-Gly increased as the ascorbate concentration in the reaction increased (K_m=28μM at 0.625mM ascorbate). Addition of 100μM Ac-ACTH(1-14) or γ_2MSH inhibited the reaction: addition of several α-amidated peptides (100μM) had no effect. Supported by AM-19859, AM-18929 and Upjohn.

329.14 CHARACTERIZATION OF A BOVINE CHROMAFFIN GRANULE ENKEPHALIN PROCESSING ENZYME C.M. Troy* and J.M. Musacchio (spon. M. Puig). Dept. of Pharmacology, N.Y. Univ. Med. Ctr. New York, NY 10016.

Bovine chromaffin granules are an excellent system for studying the biosynthesis of enkephalins because they contain both methionine and leucine enkephalins, and their precursors. We have previously reported the detection of enzyme activities in the chromaffin granules which cleave enkephalins from larger polypeptides (Life Sci. 31:1717-1720, 1982). We have further characterized an enzyme activity which cleaves leucine enkephalin (LeuE) from peptide E.

In our recent studies, radiodinated peptide E was utilized as the substrate to detect enkephalin processing enzyme activity. Unambiguous identification of [^{125}I]-LeuE as the major digestion product was effected by a combination of HPLC and TLC. Chromaffin granule lysates were incubated with the substrate and then chromatographed, along with cold I-LeuE, on an Altex RP-18 column. The radioactive fractions corresponding to the peak of I-LeuE were further analyzed by TLC and visualized by autoradiography; spots coinciding with the I-LeuE were scraped and counted, allowing precise quantification of the [^{125}I]-LeuE formed.

Studies with protease inhibitors indicate that the enzyme activity producing [^{125}I]-LeuE is due to a thiol protease with specificity for arginyl residues. PCMB completely inhibits the cleavage and this inhibition can be reversed by the addition of DTT, a sulfhydryl reducing agent. The microbial derived protease inhibitors, leupeptin and antipain, both inhibit the processing. These inhibitors act best on thiol proteases, especially those enzymes cleaving at the carboxyl side of an arginine residue. Our previous studies have shown that optimal activity occurs at the intragranular pH of 5.5-6.0.

Preincubation of the chromaffin granule lysates results in an enhancement of the enzyme activity. The mechanism of this enhancement was investigated and preliminary results indicate that it is due to the activation of a proenzyme; possibly by autoactivation or cleavage by another protease.

It is proposed that the converting enzyme in the bovine chromaffin granules is a unique intracellular protease which has the characteristics of an acid thiol protease with a cleavage specificity for basic amino acid residues, specifically arginyl residues. This protease may exist in the granules as a proenzyme which requires activation, possibly accounting for the small amount of processing seen in vivo. This work was supported in part by PHS grants DA02013, MH17785 and MH29591; CMT was supported by MSTP grant GM07038.

329.15 EFFECT OF HALOPERIDOL TREATMENT ON PROENKEPHALIN mRNA CONTENT OF RAT BRAIN. I. Mocchetti*, E. Costa, J.P. Schwartz (SPON: M.Henkart), Lab. Preclinical Pharmacology, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032

Daily administration of haloperidol increases met^5-arg^6-phe^7-, met- and leu-enkephalin content of striatum suggesting that the biosynthesis of enkephalins is increased. In order to elucidate the mechanisms operative in this increase, proenkephalin mRNA was measured with the use of a cDNA probe for human pheochromocytoma proenkephalin (Comb et al., Nature 295:663, 1982). Total RNA was extracted from tissues with guanidine thiocyanate and purified on a cesium chloride gradient. Poly $(A)^+$ RNA (mRNA) was then prepared by two passages over oligo (dT)-cellulose. The mRNA was size-separated on a formaldehyde-agarose gel, blotted to nitrocellulose paper and hybridized with a 918 bp fragment of the cDNA. Quantitation was done by densitometric scanning of the autoradiograms. A single mRNA species, approximately 1400 bases, was detected in each of seven brain regions; striatum, hypothalamus, cortex, cerebellum, midbrain, brain stem and hippocampus. The cross-reactivity with the probe for human pheochromocytoma proenkephalin suggests that brain proenkephalin has a similar sequence and furthermore that human and rat are similar. Long-term administration of haloperidol (1 mg/kg daily for three weeks) led to an increase in proenkephalin mRNA in striatum (2.6 fold) and in midbrain (5.0 fold) with no change in any other brain region. A study of the time required for the haloperidol effect showed that following four days of treatment, the increase in striatum was essentially maximal. Reserpine treatment (0.5 mg/kg for two days: animals sacrificed five days later) resulted in a doubling of striatal proenkephalin mRNA. Neither treatment changed total mRNA content. Our results suggest that dopamine may exert a tonic inhibition on proenkephalin mRNA synthesis: removal of the inhibitory influence by either haloperidol or reserpine results in increased transcription of the proenkephalin gene. An increased synthesis and turnover of enkephalin peptides may participate in the therapeutic profile of dopamine receptor antagonists.

329.16 PRO-OPIOCORTIN-CONVERTING ACTIVITY FROM BOVINE NEUROSECRETORY GRANULES IS A GLYCOPROTEIN. D.C. Parish* and Y. P. Loh, Section Functional Neurochem., Lab. Develop. Neurobiology, NICHD, NIH, Bethesda, MD 20205.

Neuropeptides and peptide hormones are generated by cleavage from larger precursors at paired basic amino acid residues. An enzymatic activity capable of cleaving specifically at paired basic residues of pro-opiocortin (ACTH/endorphin precursor) has previously been described in bovine neurosecretory granules (Chang et al., Endocrinology 111: 1607-1614, 1982) In that study bovine neural lobe granules were purified, lysed, and the supernatant was chromatographed on Sephadex G75. The enzyme activity was assayed by incubating the column fractions with [^{3}H] arginine or [^{3}H] phenylalanine labelled toad pro-opiocortin (obtained by incubating neurointermediate lobes from Xenopus laevis with labelled amino acids). The products generated from the pro-opiocortin by the converting activity were separated on acid urea polyacrylamide gels and identified by immunoprecipitation. Incubations at pH5 yielded the following products: 21K ACTH, 21K ACTH/LPH, 16K glycopeptide, 13K ACTH, β-LPH and β-endorphin-like peptide.

This work was extended by examining the ability of the pro-opiocortin converting enzyme activity to bind to concanavalin A. The fraction from Sephadex G75 which contained the highest converting activity was passed down a column of Concanavalin A covalently linked to Sepharose 4B, in a buffer of 10 mM Tris Cl, 0.7 mM $MgCl_2$ and 1M NaCl at pH 7.4. After three washes with this buffer, the column was treated with four more washes using the same buffer which included 0.2 M α-methyl D mannoside, thus eluting any glycosylated material. The fractions from this column were incubated with [^{3}H] toad pro-opiocortin and the radioactive products examined by acid-urea polyacrylamide gel electrophoresis. Activity from three separate Sephadex G75 columns was examined in this way and in each case the enzyme bound to concanavalin A and was eluted by α-Methyl D Mannoside. The pattern of [^{3}H] products seen on acid-urea gels was identical to that seen in the material before separation on the concanavalin A column. 90% of the protein applied to the Concanavalin A column (as measured by Lowry protein assay) did not bind to it, so that a major purification of the activity had been effected. This purification was confirmed qualitatively by SDS polyacrylamide gel electrophoresis.

Therefore it is concluded that pro-opiocortin converting activity in the bovine neurosecretory granule is a glycoprotein. This procedure will be used as a step in the further purification of the pro-opiocortin converting enzyme from neural lobe secretory granules.

329.17 MODULATION OF PREPROENKEPHALIN A MESSENGER RNA ACTIVITY IN RAT STRIATUM BY HALOPERIDOL AND LITHIUM CHLORIDE. K.Yoshikawa[1], S.L.Sabol[2], and J.S.Hong[1]. [1]Lab. Behav. Neurol. Toxicol., NIEHS/NIH, Research Triangle Park, NC 27709, [2]Lab. Biochem. Genetics, NHLBI/NIH, Bethesda, MD 20205

Repeated administration of haloperidol (HAL) or lithium chloride (Li) increases the levels of methionine-enkephalin (ME) in the rat striatum and nucleus accumbens. Since these psychoactive compounds exert an inhibitory effect on the dopaminergic transmission, it is likely that striatal enkephalinergic neurones are under a tonic inhibitory influence of dopaminergic neurones. In order to estimate the biosynthetic rate of ME after repeated administration of HAL and Li, we determined the striatal level of mRNA coding for ME precursor molecule using an in vitro translation method.

Male Fischer 344 rats were injected with HAL (2 mg/kg/day, s.c. for 21 days) or Li (5 meq/kg/day, i.p. for 5 days). Cellular RNA was extracted from the striatum by the method of Chirgwin et al. (Biochemistry, 18, 5294 (1979)). Poly(A)+ RNA (mRNA) was purified by chromatography on poly(U)-Sepharose and translated in the rabbit reticulocyte system supplemented with L-(^{35}S)-methionine. (^{35}S)-labeled ME precursor was purified by immunoprecipitation with the affinity purified IgG against ME-Arg^6-Phe^7 and analysed by fluorography after SDS-polyacrylamide gel electrophoresis.

Repeated administration of HAL and Li caused an 86% and 66% increase, respectively, in the striatal level of ME. The electrophoretic analysis of the immunoprecipitates revealed four proteins, having apparent Mr values of 31,000, 30,000, 22,000 and 20,000. The protein with Mr 30,000, presumably preproenkephalin A, exhibited the most intense fluorogram among these proteins. Translation of mRNA from the HAL-treated rats resulted in a 106-108% increase in the rate of (^{35}S)-methionine incorporation into the precursors as compared with the equal amount of mRNA from the control animals, whereas translation of cellular RNA (unfractionated RNA) from the HAL-treated rats revealed a 44% increase in the synthesis of (^{35}S)-preproenkephalin with Mr 30,000. On the other hand, translation of mRNA from the Li-treated rats resulted in a 26% increase in the synthesis of Mr 30,000 + 31,000 (^{35}S)-preproenkephalin compared with the equal amount of mRNA from the control rats.

These results suggest that HAL elevates the striatal ME content primarily by increasing the precursor mRNA activity. In contrast, Li elicits a relatively small increase in striatal preproenkephalin A mRNA content while eliciting a larger increase in ME content. This discrepancy suggests that an elevation of biosynthesis may not be the sole mechanism for Li-elicited elevation in ME content.

329.18 A PEPTIDASE THAT CLEAVES ENKEPHALIN ANALOGOUSLY TO BRAIN ENKEPHALINASE IS PRESENT IN TORPEDO ELECTRIC ORGAN MEMBRANES. Miriam Altstein*, Zvi Vogel and Yadin Dudai. Department of Neurobiology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Membrane-bound proteases have been implicated in the biosynthesis, maintenance and function of cell membranes, and in regulating the availability of extracellular peptidergic signals. Torpedo electric organ, which contains large quantities of homogeneous cholinergic synapses, may serve as a suitable model system for the molecular and physiological investigation of proteases in nerve and muscle membranes. Short peptides can be used as sensitive substrates for the the detection and characterization of such enzymes. Employing [^{3}H]-Leu-enkephalin as a substrate, we have detected proteolytic activity in membrane fractions prepared from the electric organ of Torpedo californica. Part of the proteolytic activity was associated with the crude mitochondrial fraction, i.e. the fraction containing synaptic membranes, even after extraction in high salt followed by hyposmotic shock and washing. The majority of the enkephalin-degrading activity could be attributed to an enzyme that cleaves the peptide at the Gly^3-Phe^4 bond. The activity ($K_m \sim 4 \times 10^{-5}$M) was completely inhibited by 10μM of thiorphan or phosphoryl-Leu-Phe but was not affected by the aminopeptidase inhibitors puromycin and bestatin (100μM and 10μM, respectively). The specificity of cleavage and inhibition of the Torpedo enzyme thus resembles that of enkephalinase, a Zn^{+2}-metalloenzyme which seems to play a role in the degradation of enkephalins in mammalian brain. Several methods of subcellular fractionation were employed to further characterize the enzyme. On a steep sucrose-density flotation gradient, the majority of the proteolytic activity comigrated with α-bungarotoxin-binding sites and was distinctly separated from acetylcholinesterase-bearing particles. On a shallow sucrose-density sedimentation gradient, most of the proteolytic activity was separated from the membranes enriched in the nicotinic receptor, although a small fraction still cosedimented with the latter. On such a gradient, the peptidase separated into 4-6 peaks but no major differences were detected between the peaks with respect to the specificity of cleavage of the enkephalin molecule. Thus it appears that the peptidase is associated with several types of membrane subfractions. (Supported by The Israeli Academy of Science).

329.19 THE DISTRIBUTION AND MOLECULAR NATURE OF PROENKEPHALIN-IMMUNOREACTIVITY IN THE BOVINE BRAIN. D. Liston[1], P. Böhlen[2] J.J. Vanderhaeghen[3] and J. Rossier[1]. [1]CNRS Physiologie Nerveuse 91190 Gif-sur-Yvette, France [2]The Salk Institute, San Diego [3]The University of Brussels, Belgium.

The primary sequence of adrenal proenkephalin (PE) has recently been deduced from the nucleotide sequence of the cDNA for this protein. The complete precursor contains 6 copies of Met-enkephalin (ME) and one copy of Leu-enkephalin (LE). Numerous intermediates in the processing of PE have been purified from the adrenal medulla, but similar intermediates have not been isolated from brain. To examine the possibility that the brain employs a similar precursor, we have used a radioimmunoassay (RIA) which recognizes the N-terminal region of PE 1) to determine the molar ratio of PE immunoreactivity (PE-IR) to ME-IR in various brain regions and 2) to examine the molecular nature of the PE-IR in the brain. ME-IR and PE-IR were determined on acetic acid extracts of bovine brain using specific RIAs. High concentrations of PE-IR were present in the brain areas which are rich in ME, in particular the globus pallidus (1098±291 fmol/mg tissue), caudate (375±17 fmol/mg), putamen (256±34 fmol/mg) and hypothalamus (124±14 fmol/mg). Assay of ME after digestion with trypsin and carboxypeptidase B (CPB) yielded a molar ratio of ME-IR to PE-IR in these tissues ranging from 4.3 to 12.5. In most areas the PE-IR elutes as a single species on exclusion chromatography and does not yield ME or LE on digestion with trypsin and CPB. We have purified this protein (synenkephalin) to homogeneity from bovine caudate nucleus. Amino acid analysis allows us to conclude that it represents residues 1-70 of PE. In contrast to the other brain regions examined, the hypothalamus contains several PE-IR species which yield ME upon enzymatic digestion. We have exploited the anatomical features of the hypothalamo-neurohypophyseal system to study more closely enkephalin biosynthesis in these neurons. In the cell bodies of the supraoptic nucleus, 75% of the total ME resides in extended peptides with molecular weights as great as 20 kDaltons. In the axons of the pituitary stalk these larger ME-containing peptides represent 56% of the total ME. Terminals in the posterior pituitary contain only 11% of the total ME in larger peptides. Thus, the relative proportions of the larger precursors decreases progressively from the cell bodies to the terminals, with an accumulation in the terminals of the free enkephalins and synenkephalin, the N-terminal fragment of PE. We conclude that the brain and the adrenal gland utilize a similar pathway for enkephalin biosynthesis, and that processing of PE in the hypothalamo-neurohypophyseal neurons occurs concurrently with axonal transport of the prohormone.

(Supported by The Esther A. and Joseph Klingenstein Fund, N.Y.)

EFFECTS OF DRUGS ON RECEPTORS

330.1 THE ACTIONS OF n-ALKANOLS ON MINIATURE END-PLATE CURRENTS. J. G. McLarnon*, P. Pennefather* and D. M. J. Quastel. Department of Pharmacology, Faculty of Medicine, The University of British Columbia, Vancouver, B. C., V6T 1W5, Canada.

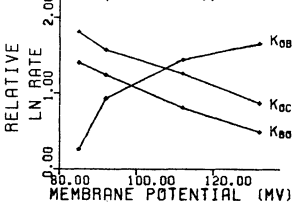
At the mouse end-plate, n-alkanols act to modify the amplitude and time course of miniature end-plate currents (MEPCs). Hexanol, heptanol, and octanol all cause two-component MEPC decays similar to those seen with local anaesthetics and a variety of other "non-specific drugs". The inflection point between the fast and slow components becomes lower with increasing chain length and with increasing concentration. The interaction between these agents and end-plate channels fits a sequential model, equivalent to "uncompetitive" inhibition, and onward (channel blocking) and off (channel unblocking) rate constants can be obtained from the time course of MEPCs. The onward rate constant is highest for the longest chain length member studied, octanol, and diminishes about 2-fold/methylene group, as chain length is decreased. The off-rate constant is lowest for octanol and increases progressively, about 2-fold/methylene group as chain length is decreased. With heptanol and octanol, both rate constants are temperature-sensitive, with an apparent activation energy higher for the "on" than for the "off" rate constant. The intermediate chain length alkanols (pentanol, butanol and propanol) cause prolonged MEPCs of lower than normal amplitude, with no division into two components. At 15°C, however, pentanol causes a biphasic MEPC with rate constants in accord with predicted values extrapolated from the longer chain length alkanols. The relation of kinetic rate constants to chain length suggests that the intermediate and long chain alcohols interact in the same way with the ACh-receptor-ionophore: the systematic modulation of rate constants with chain length is consistent with channel blockade being secondary to hydrophobic bonding. Thus octanol, the most hydrophobic agent of the group studied, has the highest onward rate constant and is also stabilized at the site of interaction for the longest time. The variation in rate constants, from agent to agent, is indicative of a specific interaction of the n-alkanols with a hydrophobic region of the protein associated with the end-plate channel and extrapolation of the observed rate constants to the intermediate chain length n-alkanols predicts monophasic MEPCs, with prolonged decays and reduced amplitudes, as is observed. To extend the model to methanol and ethanol, which prolong MEPCs without reducing MEPC amplitude, it is necessary to postulate that when these agents interact with the receptor the channel remains open and permeable to cations, although, as with the higher chain alkanols, it is incapable of relaxing to the normal closed state until the n-alkanol molecule leaves its binding site.

330.2 KINETICS OF NICOTINIC ION CHANNEL BLOCKADE. Anthony Auerbach* (SPON: C. Lingle). Dept. Biophysical Sciences, State Univ. New York, Buffalo, NY 14214.

Many cationic ligands can both activate and block nicotinic ion channels. I have studied the kinetic properties of single nicotinic channels influenced by two such agents, benzyltrimethylammonium (BzTMA; 2-20 uM) and trimethylphosphonium (TMP; 1 mM). Pectoral muscles from 12-13 day chick embryos were grown for 1-2 weeks in tissue culture. Data were from cell-attached patches at 22-24 °C.

In the presence of BzTMA, channel currents occurred in bursts. The amplitude of virtually all gaps within bursts was zero. The results from one patch are shown below, where event durations from 50 pS channels were analysed according to a three state (closed-open-blocked) sequential model. The closing rate (k_{oc}) and the unblocking rate (k_{bo}) each showed simple exponential voltage dependence and decreased e-fold with 50 mV hyperpolarization. The blocking rate (k_{ob}) increased with hyperpolarization in a non-exponential manner, saturating at extreme potentials. At a membrane potential of -120 mV the estimated rate constants were: k_{oc}=160 sec^{-1}, k_{bo}=9700 sec^{-1}, and k_{ob}=7x10^7 $sec^{-1}M^{-1}$. For 35 pS channels, the closing and unblocking rates were similar to the 50 pS values but the blocking rate was 2-4 times slower. The saturation of the blocking rate with voltage does not appear to be due to unresolved openings or to permeation of the blocker through the channel. The results can be explained by a two step blocking process, one step of which becomes rate limiting at hyperpolarized potentials.

TMP-activated channels appeared as rectangular pulses with few gaps. The mean channel lifetime increased with hyperpolarization and was 3.8 msec at -140 mV. With hyperpolarization, the I/V relation of the unitary current became sublinear and the variance of the open channel current noise increased markedly. At a bandwidth of 2 kHz and at -140 mV, the unitary current was 2.5 pA and the open channel variance, 0.52 pA^2 (compared to 0.23 pA^2 for the baseline). Spectral analyses of the open channel noise should reveal if these data are consistant with an open-blocked kinetic scheme where the blocking rate is diffusion-limited and the unblocking rate is greater than 10^3 sec^{-1}.



Supported by NINCDS 13194 to Frederick Sachs.

330.3 CONOTOXIN G_1 BLOCKS ACH BINDING TO ITS RECEPTOR AT THE FROG END PLATE. O. B. McManus* and J. R. Musick. Department of Physiology, University of Utah School of Medicine, Salt Lake City, Utah 84108.

Conus geographus is a marine snail with a well developed venom apparatus that it uses both defensively and in preying on small fish. A small peptide (Conotoxin G_1) has been isolated from the crude venom, characterized, and shown to cause death when injected into mice, by flaccid paralysis and respiratory failure (Gray, et.al.,J.Biol.Chem.,256, 1981). These studies were done on frog cutaneous pectorus muscles with a synthetic form of Conotoxin G_1 provided by J.Rivier of the Salk Institute.

Conotoxin G_1 reversibly blocks nerve evoked contractions of frog muscle at 3-4 micromolar concentration. Earlier experiments showed that the toxin has no effect on the amplitude of directly stimulated muscle contractions (McManus, et.al.; Neurosci.Lett.,24, 1981). Micromolar concentrations of Conotoxin G_1 significantly reduce the amplitude of both miniature endplate potentials (mepps) and membrane depolarizations resulting from ionophoretic application of acetylcholine (ACh). These reductions in mepp and ionophoretic potential amplitude were not due to changes in muscle fibre resting membrane potential or input resistance. These results suggest that Conotoxin G_1 blocks neuromuscular transmission at a post synaptic site.

The effects of Conotoxin G_1 on the ACh receptor at the neuromuscular junction were investigated using focal extracellular recording of endplate currents (epcs) and miniature endplate currents (mepcs). Conotoxin G_1 reversibly reduced the amplitude of mepcs and epcs, but had no significant effect on the rate of decay of mepcs or epcs. These results suggest that Conotoxin G_1 does not affect the lifetime of ion channels open by ACh. When acetylcholinesterase is blocked, mepcs decay five to six times more slowly than normal, with a rate that is determined by the rate of diffusion of ACh from the synaptic cleft. This rate of diffusion is slowed by repeated binding of ACh to receptors as it diffuses from the cleft, and increased by drugs that reduce ACh binding to its receptor (Katz and Miledi, J. Physiol.231, 1973). Conotoxin G_1 reduced the amplitude and increased the rate of decay of mepcs recorded at prostigmine treated endplates. These results suggest that Conotoxin G_1 reduced binding of ACh to its receptor at the frog endplate. Conotoxin G_1 may be a useful probe of ACh receptor function.

330.4 EFFECTS OF CHLORAMPHENICOL STEREOISOMERS ON ENDPLATE CHANNELS F. Henderson* and I.G. Marshall* (SPON: N.N. Durant) Dept. Physiol. & Pharmacol. Univ. of Strathclyde, Glasgow G1 1XW, UK.

A wide range of structurally dissimilar compounds interact with the open form of the acetylcholine receptor-activated ion channel, resulting in an alteration of endplate current (EPC) decay. The lincosamide antibiotics have been shown to act in this manner (Fiekers, J.F. et al., Nature, 281:680, 1979) and exert their antibacterial action by binding to the 50S subunit of bacterial ribosomes. Chloramphenicol (D-threo) also binds to the 50S subunit and competes with the lincosamides for this binding site. The present experiments were designed to assess the relationship between antibacterial and ion channel blocking activities of chloramphenicol. The optical isomer of chloramphenicol (L-threo) which possesses less than 2% of the antibacterial activity of the D-threo form, was also studied to assess any stereospecificity of action of the compounds at the ion channel. The effects of the compounds on EPC decay characteristics were tested at 20-22°C in the voltage clamped cut costocutaneous nerve-muscle preparation of the garter snake. Both chloramphenicol analogs (2×10^{-4}-10^{-3}M) split EPC decays into two components, one faster and one slower than the control decay rate. The effects on the two components were both concentration- and voltage-dependent. Thus, increasing the concentration produced a progressive increase in the decay rate of the fast component and a decrease in the decay rate of the slow component.

		τ_{fast} (msec)		τ_{slow} (msec)	
CONCENTRATION (M)		D-threo	L-threo	D-threo	L-threo
2×10^{-4}	-110mV	0.47 ±0.05	0.45 ± 0.02	4.04 ± 0.33	4.57 ± 0.41
5×10^{-4}	-110mV	0.39 ±0.01	0.35 ± 0.02	10.2 ± 0.3	11.2 ± 0.9
10^{-3}	-110mV	0.31 ±0.01	0.30 ± 0.03	26.4 ± 3.6	20.5 ± 2.0

The decay of the fast component showed little dependence on voltage, but the voltage-dependence of the slow component was slightly greater than that of control.

It is concluded that the chloramphenicol analogs interact with the endplate ion channel. Despite the stereospecificity of the antibacterial action of chloramphenicol, no such stereospecificity could be demonstrated at the endplate neuromuscular junction. This lack of stereospecificity suggests that the channel blocking process is diffusion-limited.

Supported by MRC, Leverhulme, Wellcome, SKF Foundation and Parke-Davis.

330.5 THE ANTICHOLINESTERASE AGENT, PHYSOSTIGMINE (PHY), BLOCKS THE IONIC CHANNEL OF THE NICOTINIC RECEPTOR IN ITS OPEN CONFORMATION. K.P. Shaw*, A. Akaike* and E.X. Albuquerque (SPON: C. Spivak). Dept. Pharm. & Exp. Ther., Univ. Maryland, Sch. Med. Balt., MD 21201.

The carbamate, PHY, is a well-known reversible inhibitor of acetylcholinesterase. Recent evidence from our laboratory indicates that the molecular target of some anticholinesterase agents, such as pyridostigmine (PYR) is the acetylcholine receptor-ionic channel complex (AChR).. PYR is a weak agonist of the AChR. In patch clamp studies, PYR produces high frequency channel openings, 'flickering' and 'bursting' of ACh-activated channels, followed by a silent state suggestive of desensitization. Channel conductance is also reduced, but channel lifetime is unaltered (Akaike et al., unpublished results). In this study we attempt to identify the effect of PHY and the organophosphate agent, sarin, on the AChR of the frog sartorius muscle. PHY (10 μM) depressed the indirectly evoked muscle twitch tension to less than 50% of control values. PHY (0.1-1 μM) increased peak endplate current (EPC) amplitude by 17-26% and prolonged the EPC decay time constant (τ_{EPC}) by 18-68% at -90 mV. At higher concentrations, PHY (10-100 μM) decreased peak EPC amplitude by 18-19% and shortened the τ_{EPC} by 23-67% at -90 mV. τ_{EPC} was shortened to a greater extent at negative potentials. The current-voltage relationship for peak EPC amplitudes (from -150 to +60 mV) in the presence of PHY was linear as well as the plot of $1/\tau$ vs PHY concentration. PHY (1 μM) increased peak miniature EPC (MEPC) amplitude and τ_{MEPC}, while at 100 μM the MEPC amplitude and τ_{MEPC} were decreased. Fluctuation analysis of ACh-induced EPCs showed that PHY (10 μM) shortened the single channel lifetime (τ_{noise}). Sarin (10 μM) increased peak EPC amplitude by 20% and prolonged τ_{EPC} by 25%. After washing, a significant increase of peak EPC amplitude as well as τ_{EPC} was observed. Exposure to PHY (0.1-33 μM) after complete inhibition of acetylcholinesterase with sarin, decreased peak EPC amplitude by 30-50% and shortened τ_{EPC} by 25-65%. Enhancement of EPC amplitude as seen in the presence of low concentrations (0.1-1 μM) of PHY alone was not observed when acetylcholinesterase was irreversibly inhibited by sarin. PHY (1-33 μM) shortened the channel lifetime in a concentration-dependent manner but did not alter the channel conductance as studied by the patch clamp technique. The shortened channels appeared to be clustered. In contrast to PYR, which interacts directly with ACh recognition sites, PHY appears to act as an open channel blocker. (Supported by U.S. Army Medical Research and Development Command contract DAMD 17-81-C-1279.)

330.6 DIISOPROPYLFLUOROPHOSPHATE ALTERS NICOTINIC TRANSMISSION IN NG108-15 - MYOTUBE CO-CULTURES. M. Adler, G.J. Pascuzzo*, D. Maxwell*, J.F. Glenn and R.E. Foster. Neurotoxicology Branch, U.S. Army Med Res. Institute of Chem. Defense, APG, MD 21010.

The actions of the irreversible organophosphorous cholinesterase (ChE) inhibitor, diisopropylfluorophosphate (DFP), were investigated on passive electrical properties, action potential generation, and synaptic transmission in co-cultures of NG108-15 neuroblastoma x glioma hybrid cells and clonal G8-1 myotubes. Co-cultures were maintained in DMEM supplemented with 10% horse serum, 100 μM hypoxanthine, 16 μM thymidine and 1 mM dibutyryl cyclic-AMP. Intracellular recordings were performed at 35°C in standard physiological solution 5-7 d after myotube cultures (20% confluent) were seeded with 50,000 NG108-15 cells. Approximately 30% of the myotubes were innervated as indicated by the presence of spontaneous miniature synaptic potentials (MSPs), which represent quantal release of acetylcholine from NG108-15 cells. Under control conditions, MSPs had a rise time of 4.41 ± 0.29 msec and occurred at a frequency of 17.2 ± 2.1/min (n=9 myotubes). MSPs were generally biphasic consisting of a large depolarizing component (5.62 ± 0.85 mV) followed by a smaller hyperpolarizing component. Both components were blocked by 2 μM d-tubocurarine. The depolarizing phase had a predominantly exponential decay with a half time of 4.78 ± 0.28 msec. Low concentration of DFP (1-10 μM) produced a 2-fold increase in the MSP frequency but did not change its amplitude. In the presence of 100 μM DFP, reductions were noted in both MSP frequency (48%) and amplitude (35%). Raising the DFP concentration to 1 mM resulted in further reductions in MSP frequency (90%) and amplitude (50%). These actions of DFP were rapidly reversible by washing cells in drug-free solution. The MSP time course underwent small concentration-dependent increases following exposure to DFP. In concentrations up to 1 mM, DFP had little or no effect on myotube or NG108-15 resting potential, input resistance and time constant. NG108-15 Na^+ and Ca^{2+} spikes exhibited small reductions in amplitude and maximum rates of rise after addition of 1 mM DFP. ChE activity of G8-1 myotubes was found to be 0.062 nmoles ^{14}C-acetylcholine hydrolyzed/min/mg protein. By use of specific inhibitors, BW-284C51 and iso-OMPA, 83% of activity was determined to be acetyl-ChE whereas 16% was butyryl-ChE. These ratios are similar to those observed in adult mammalian skeletal muscle. The relatively low ChE activity of the G8-1 cell line is consistent with our observations that DFP does not potentiate synaptic transmission appreciably in these co-cultures. Thus, the NG108-15/G8-1 co-culture may be a good model for studying the direct effects of organophosphorous ChE inhibitors. (Supported under USAMRICD Protocol #1-03-82-000-A-216).

330.7 PHARMACOLOGIC DIFFERENCES BETWEEN SLOW AND FAST NEUROMUSCULAR SYSTEMS. R.J. Storella, Jr.*†, W.F. Riker and T. Baker. Dept. of Pharmacology, Cornell Univ. Medical College, New York, NY 10021.

The neuromuscular blocking drug d-tubocurarine (DTC) was used to probe for differences between fast and slow neuromuscular systems. Male Sprague-Dawley rats were anesthetized with urethane (1.6 g/kg). The soleus (SOL, a slow neuromuscular system) and/or the tibialis anterior (TA, a fast neuromuscular system) were prepared for recording isometric contractile tension and/or unipolar electromyogram. The muscles were stimulated via the sciatic nerve.

At low frequencies of stimulation (0.2 and 1.0 Hz) the TA was more sensitive to DTC neuromuscular block than was the SOL. In both neuromuscular systems, the sensitivity to DTC increased with increasing frequency of stimulation.

When doses of DTC which minimally reduce transmission at 0.2 Hz preceded a tetanus at 50 Hz, there was an abrupt and progressive decline of the transmitted response during the tetanus. This phenomenon is termed tetanic fade. Fade in the fast TA system was greater than in the slow SOL system.

Following tetani of 25, 50, 100 and 200 Hz for 5 seconds, there was a transient reversal of an 80% DTC block in both fast and slow systems responding to indirect stimulation at 0.2 Hz. The extent of the decurarization depended upon the frequency of the tetanic stimulation. In this regard, the TA system was more responsive to decurarization than was the SOL system.

It is concluded that the differences in the responsiveness of slow and fast neuromuscular systems to DTC relate to different physiologic characteristics of these systems. The differences in DTC caused tetanic fade and frequency dependent decurarization between the slow and fast neuromuscular systems indicate that DTC action on motor nerve terminals is involved in the transmission block.

† Supported by NIGMS Training Grant No. GM-07547.

330.8 EXTERNAL AND INTERNAL PERFUSION OF GABA AND ITS ANTAGONISTS IN THE SINGLE FROG PRIMARY AFFERENT NEURON, N. Akaike*, K. Hattori* and Y. Oomura., Dept. of physiology, Fac. of Medicine, Kyushu Univ. 60, Fukuoka 812, Japan.

The Cl currents in the frog dorsal root ganglion cells were separated from Na, K and Ca currents by using suction pipette technique which allows internal perfusion and voltage clamp. Cl^- current was separated after Na^+, Ca^{++} and K^+ currents were blocked by the substitution of Tris for Na^+, Mg^{++} for Ca^{++} and Cs^+ for K^+ in both external and internal solutions. The membrane potential of neuron was measured using an Ag-AgCl wire mounted into the Ringer-agar plug which was placed inside the suction pipette. The reference electrode was an Ag-AgCl wire connected to bathing medium through Ringer-agar bridge. The resistance between the suction pipette with 10 to 11 μm tip and the bath electrode was 400 KΩ in Ringer solution. Both electrodes were led to a single electrode amplifier circuit with switching system to record the potential change and to inject constant current pulse. The dose-response curves to GABA were investigated in the presence of external and internal application of GABA antagonists. With external application of antagonists, the inhibition of the GABA-induced Cl currents by bicuculline, strychinine, curare, atropine, D-600 and SHIT was competitive, that by picrotoxin was noncompetitive, while that by penicilline was uncompetitive. Only picrotoxin which was applied from either side of the cell membrane blocked GABA and pentobarbital (PB) responses. Picrotoxin blocked the GABA- and PB-induced single Cl channel currents in the inside-out patch clamp preparations with shortening of lifetime of single Cl channel current. In addition, the competitive blockers suppressed GABA- or PB- induced response without changing the slope of a ramp current-voltage relationship while the inhibition by a noncompetitive blocker, picrotoxin was followed by the change of slope.

The results suggest that picrotoxin has a direct action on the Cl channel while competitive drugs act on the receptor site.

330.9 ACTIONS OF SUBSTANCE P ON RAT SPINAL DORSAL HORN NEURONS. K. Murase and M. Randić. Biomed. Eng. and Dept. of Vet. Physiology and Pharmacology, Iowa State University, Ames, IA 50011.

The membrane actions of substance P (SP) and the effects on calcium-dependent action potential of dorsal horn neurons have been investigated by means of intracellular recording techniques in the immature rat in vitro spinal cord slice preparation (J. Physiol. 334:141-153, 1983).

Bath-application of SP (2×10^{-6} to 10^{-5} M) induced a biphasic membrane response consisting of an initial hyperpolarization followed by a depolarization in about one-third of the examined cells. Initial hyperpolarization was not observed when synaptic activity was blocked by perfusing the slice with a TTX-, or a Ca^{2+}-low, Mg^{2+}-high Ringer solution. This result is consistent with a presynaptic action of SP mediated through excitation of inhibitory interneurons. This interpretation was supported by recording of repetitive spontaneous IPSP-like hyperpolarizing potentials during the initial hyperpolarization.

When Co^{2+} was used to block voltage-dependent Ca conductance and possible indirect presynaptic actions, SP induced only a small depolarization. When TTX was used, SP-induced increase in neuronal input resistance was not modified, although depolarization was slightly diminished. In contrast, in TTX + TEA-containing medium, the SP depolarizing response was enhanced and accompanied by a small decrease in input resistance and firing of Ca-spikes. These results suggest that SP-induced depolarization might be a consequence of a reduction in a voltage-dependent potassium conductance allowing Na and/or Ca conductances to dominate.

SP modified duration of Ca-dependent action potentials of dorsal horn neurons, the most consistent change being an initial dose-dependent and reversible decrease of the spike duration. The decrease in the Ca-spike duration was associated with a small reduction in the rate of rise and peak amplitude, and significant parallel increase in dV/dt of the falling phase of Ca-spike. Our data indicate that the initial decrease in Ca-spike duration was not due to the depolarizing action of SP, although shunting of the membrane resistance, either through presynaptic or postsynaptic mechanisms has not been ruled out. Alternatively, these data are consistent with a possibility that SP shortens the duration of the Ca-spike by decreasing a voltage-sensitive inward Ca-current and/or augmenting an outward potassium current(s). The direct test of our interpretation is to be accomplished only with a voltage-clamp analysis.

This work was supported by NIH grant (NS 17297) and the United States Department of Agriculture.

330.10 DOSE-DEPENDENT EFFECTS OF ETHANOL ON GABA RESPONSES AND IPSPs MEASURED INTRACELLULARLY IN HIPPOCAMPAL CA1 CELLS, P.L. Carlen, N. Gurevich* and M.F. Davies*. (SPON: M.P. Charlton). Addiction Research Foundation Clinical Institute & Playfair Neuroscience Unit, Toronto Western Hospital, Depts. of Medicine & Physiology, University of Toronto, Ontario, Canada

Ethanol has been shown to enhance calcium-mediated potassium conductance and not GABA action at low doses (≤ 20mM) in hippocampal CA1 cells (Carlen, Gurevich, Durand, Science, 215: 306-309, 1982). IPSPs and EPSPSs were also enhanced at these low doses, possibly because of enhanced presynaptic transmitter release. The interaction of different doses of ethanol with IPSPs and GABA responses have been examined further. Ethanol was either added to the perfusate or focally applied by pressure-ejection. GABA (10^{-2}M) was focally pressure ejected near the soma of a recorded neuron. K acetate (3M) electrodes were used for intracellular recordings.

Stratum radiatum evoked IPSPs in CA1 cells often have 2 discernable phases; an early phase (I) probably resulting from GABA-mediated increased Cl^- conductance, and a later phase (II) probably due to enhanced Ca^{++}-mediated K^+ conductance. IPSPs and EPSPs were measured in 34 cells using ethanol doses ranging from 5 to 200 mM. All doses tended to augment IPSPs and EPSPs. However, since the membrane usually hyperpolarized, it was often not possible to be confident that the increased EPSP height was soley due to increased presynaptic transmitter release. High doses of ethanol (50, 100, 200mM) increased phase I of the IPSP more than phase II. Low doses (≤ 20 mM) increased phase II more than phase I and sometimes decreased phase I.

Both depolarizing and hyperpolarizing GABA response were enhanced in the presence of 100 mM ethanol in the perfusate (3 cells). Following drop application of 10mM ethanol to the soma, a hyperpolarizing response in 1 cell and a depolarizing response in 2 cells were depressed.

These data suggest that ethanol augments GABA postsynaptic sensitivity at high doses and has no effect or even inhibits GABA action at lower doses. The probable enhanced presynaptic transmitter release and membrane potential changes makes the interpretation of the stratum radiatum evoked IPSP data more complicated, but the dose dependent effects of ethanol on the 2 phases of this IPSP are consistent with the measured ethanol-GABA interactions. It is suggested that mild ethanol-induced sedation could be mediated by enhanced calcium-mediated potassium conductance, and more profound intoxication or anesthesia could also include augmented GABAergic actions. (Supported by NIH RO1-NS16660-02 & MRC 6019).

330.11 EVIDENCE FOR A BLOCKAGE OF Na^{++} & K^{+} CHANNELS IN HIPPOCAMPAL CA1 PYRAMIDAL CELLS BY PHENCYCLIDINE, P.W. Kujtan*, P.L. Carlen. (SPON: P. Ashby). Institute of Medical Science, Addiction Research Foundation Clinical Institute, and Playfair Neuroscience Unit, Toronto Western Hospital, Depts. of Medicine and Physiology, University of Toronto, Ontario, Canada.

The hippocampus is considered a major site of phencyclidine (PCP) action. The purpose of this study was to investigate the effects of PCP on CA1 pyramidal neurons utilizing intracellular and extracellular recording techniques.

Male guinea pigs weighing 325-500 gms were anesthetized and the hippocampus was removed and microtomed into 450 um transverse slices which were placed in a recording chamber at 34-37°C. Micropipettes filled with 3M potassium acetate (resistance 80-180M Ω) were used for intracellular recordings. Micropipettes filled with 3M Na Cl (5-10 M Ω resistance) recorded field potentials. The Schaffer collateral and alvear pathways were stimulated using monopolar tungsten tip electrodes. PCP was applied by perfusion.

A preliminary study of orthodromically evoked responses revealed that the effects of PCP were dose dependent, enhancing field potentials (20-35%) at 0.2uM to 2.0 uM and depressing field potentials at 10uM-10mM with complete supression at 2mM. All PCP effects were found to evolve slowly (several minutes). In 29 of 31 cells exposed to 100-400uM PCP, the magnitude of the action potentials decreased, ($\bar{X} \pm$ S.D. = 29.0 ± 18 mV) and the spike width increased (75 ± 39%). Spike afterhyperpolarizations, IPSPs and EPSPs also all decreased. This was associated with an increased membrane resistance and generalized depression of spontaneous spiking, although some bursting and oscillation of the membrane potential often occurred. Cells treated with TTX (10^{-6}M) to block Na electrogenesis and synaptic activity showed,a much smaller increase in resistance, and the associated calcium spikes evoked in these cells were unaffected by PCP. PCP effects persisted in the absence of synaptic activity when slices were perfused with a solution containing no added Ca^{++} and 2.4mM Mn^{++}.

These data are consistent with a specific blockade of both Na^{+} and K^{+} conductances. It is suggested that the diminished EPSPs and IPSPs are due in large part to presynaptic inhibitory actions on axonal conduction and interneurons. The shortening and widening of the spikes and the increased input resistance are interpreted as being due to blockade of Na^{+} and K^{+} conductances. The relative lack of effect of PCP in the presence of TTX could be due to TTX preventing PCP gaining intracellular access, assuming that PCP acts from an intracellular site. (Supported by NIH R01-NS16660-02 and MRC 6019).

330.12 VOLTAGE CLAMP ANALYSIS OF AN INHIBITORY SYNAPSE IN CULTURED RAT HIPPOCAMPAL NEURONS. M. Segal and J.L. Barker, (Spon: T.G. Smith, Jr.) Lab. Neurophysiol., NINCDS, NIH, Bethesda, MD. 20205

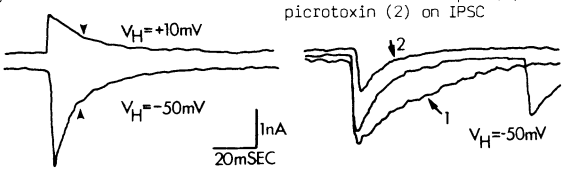
Synaptic transmission between rat hippocampal neurons grown in dissociated cell culture was studied using a two-electrode voltage clamp methodology. Action potentials in putative GABA-containing neurons were triggered by intracellular depolarizing current pulses or by extracellular pressure application of the excitant l-glutamate. Action potential discharges in presynaptic cells evoked monosynaptic inhibitory potentials in current-clamped cells or inhibitory postsynaptic currents (IPSCs) in voltage-clamped cells in more than 50 percent of the cell pairs tested. The properties of the IPSCs were compared with estimates of the single channel properties underlying responses evoked by 20 μ M GABA (derived from analysis of membrane current variance).

Property	IPSC	GABA-evoked response
Inversion Potential	-15mV	-15mV
Ion dependence	Cl^-	Cl^-
τ(IPSC decay; channel lifetime)	20.5msec	19.5msec
G(IPSC); γ(channel conductance)	29.4nS	16.7pS

On the basis of this we estimate that, on average, 1700 ion channels open simultaneously to generate the evoked IPSC and then close exponentially, oetermining IPSC decay. In some cells τ_{IPSC} and channel lifetime were significantly longer at depolarized potentials than at resting potentials (Fig. 1A). Picrotoxin (100μM) depressed the amplitude of the IPSC, but not τ_{IPSC} (Fig. 1B). The drug blocked responses to GABA without affecting channel lifetime. Diazepam (10 μM), which potentiates IPSCs and responses to GABA, slightly prolonged channel lifetime and τ_{IPSC} (Fig. 1B). Pentobarbital (100 μM) caused a threefold increase in both τ_{IPSC} and channel lifetime.

These studies demonstrate that inhibitory synaptic activity recorded in cultured hippocampal neurons appears to be mediated by GABA. Furthermore, clinically important drugs alter primarily the kinetics of GABA-activated ion channels and that may account for their effects on IPSCs. These actions may help to explain some of the therapeutic affects of these drugs in vivo.

Fig. 1 A. Inversion of IPSC B. Effects of Diazepam (1) and picrotoxin (2) on IPSC



330.13 PENTOBARBITAL PROLONGS PAIRED-PULSE POTENTIATION IN HIPPOCAMPAL CA 1 PYRAMIDAL NEURONS. M. B. MacIver* and S. H. Roth,
Department of Pharmacology & Therapeutics, Faculty of Medicine, University of Calgary, Calgary, Alberta, CANADA.

Previous studies in our laboratory have shown that pentobarbital (PB) produces biphasic alterations of synaptic transmission between Schaffer collateral fibers and CA 1 pyramidal neurons in hippocampal slices. Low concentrations (0.04 to 0.1 mM) enhanced field potential amplitudes and increased single cell discharge activity, in contrast, higher concentrations (0.1 to 0.8 mM) depressed these synaptically evoked responses. The major site of action appeared to be presynaptic and to involve calcium. Paired-pulse potentiation of this synaptic pathway is also mediated by a calcium dependent presynaptic mechanism, therefore, the effects of PB were examined on short-term potentiation.

Rat hippocampal slices (400 μm) were prepared using standard methods and submerged in a McIlwain tissue chamber (35°C, 1.0 to 5.0 ml/min). Bipolar metal stimulating electrodes were placed on Schaffer collateral fibers and used to activate synaptic inputs to CA 1 pyramidal neurons. Extracellular recording electrodes (2 M NaCl, 2 to 10 Mohm) were placed in cell body or dendritic layers of CA 1 neurons to record evoked field potentials. Paired stimulus pulses of 0.01-0.05 msec duration, 15 to 70 μA, were delivered at 0.25 Hz. Interpulse interval delays were varied between 5 and 120 msec, in 5 msec increments, to examine the time course of short-term potentiation of Schaffer collateral to CA 1 synapses. Recorded signals were amplified (x 1000), filtered (1 Hz to 10 KHz, bandpass) and averaged for later analysis.

Concentrations of PB which produced half-maximal depression of synaptic transmission (0.2 to 0.4 mM) also produced a marked prolongation of short-term potentiation. The optimal control interpulse delay time of 20 msec was shifted to 30 or 40 msec in the presence of PB, and the duration of potentiation was increased up to 100 msec. Lower concentrations (0.04 to 0.1 mM), which enhance transmission, produced a similar, but smaller increase in duration. These results agree with our previous hypothesis of a presynaptic site of action for PB.

Supported by the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research.

330.14 EFFECTS OF FOLIC ACID AND BICUCULLINE ON EVOKED RESPONSES OF HIPPOCAMPAL CA3 NEURONES IN VITRO. S. J. Kehl* and H. McLennan* (SPON: J. A. Pearson). Dept. of Physiology, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Following their treatment with convulsant agents neurones in field CA3 of the rat hippocampus discharge spontaneously in a synchronized manner with clocklike regularity. The changes in ortho- and antidromically evoked responses which accompany the development of these spontaneous population bursts (SPBs) are reported here.

Extracellular potentials were recorded in stratum pyramidale of the CA3 region (adjacent to the fimbria) of the in vitro hippocampal slice. The pre-treatment commissural (COMM) population spike (PS), evoked by a fimbrial shock had a mean latency of 2.6 msec; antidromic (AD) activation via the Schaffer collaterals in stratum radiatum of CA1 elicited a short latency (0.3-0.5 msec) PS followed by a positive wave upon which a secondary PS was superimposed. Perfusion of the slice with folic acid (10-20 ml, 10^{-3}M) or bicuculline methochloride (10-20 ml, 5-10 x 10^{-6}M) increased the amplitude of the primary PS of the COMM response. With both COMM and AD stimulation there was a progressive increase in the number and amplitude of PSs which followed the primary PS. SPBs appeared coincident with the development of evoked epileptiform firing and there was no obvious difference in the amplitude (6-15 mV) or frequency (1/5-10 sec) of SPBs induced with either compound.

With the appearance of large amplitude SPBs a marked increase in the latency of the COMM response was observed. Further analysis showed the latency to be dependent on the timing and intensity of the COMM stimulus. Thus at a constant threshold intensity the latency increased as the interval between a preceding SPB and the stimulus was decreased; at a constant SPB-stimulus interval the latency varied inversely with the intensity of the fimbrial shock. Despite these large (up to 90 msec) changes of latency observed in either situation there was no clear difference in the amplitudes of evoked epileptiform discharges which were similar to antidromically evoked and spontaneously occurring bursts.

No change in pre- and post-treatment latencies was detected when AD PSs were evoked. Latency shifts of burst discharges comparable to those observed with COMM activation were noted however if the AD stimulus intensity was decreased so as to be subthreshold for an antidromic PS.

These data imply that both folate and bicuculline enhance the electrophysiological coupling in CA3 neuronal aggregates which then behave as a functional syncytium. The physiological basis for the large shift of latency and the coupling between cells remains to be determined. Supported by the Canadian MRC.

330.15 EFFECTS OF COLCHICINE ON HIPPOCAMPAL EVOKED POTENTIALS IN VITRO. D. C. Derrington* and E. W. Lothman (SPON: E. Montgomery), Dept. Neurology, Wash. Univ., St. Louis, MO 63110.

In addition to its well-known ability to block axoplasmic transport and disrupt microtubules, colchicine has been reported to alter conductance changes and synaptic coupling in Aplysia neurons and interfere with neurotransmission at the frog neuromuscular junction. This drug has also been shown to cause paroxysmal activity and be cytotoxic to granule cells in the dentate gyrus when injected into the hippocampal formation of rats.

In order to examine the electrophysiologic action of colchicine on mammalian brain tissue we studied the effects of cochicine on hippocampus in vitro. Slices, 400μm thick, were prepared from albino rats (250-325 grams) and maintained at 37°C in a physiological buffer according to standard techniques. Glass micropipettes (5-15 MΩ, 2M NaCl) were placed in the cell body layer and apical dendrites of CA_1 pyramidal cells (n=12 slices) and granule cells of the dentate gyrus (n=7 slices) to record population spikes and population epsp's elicited at 0.2 and 1 Hz with stimulating electrodes over the Schaffer collaterals and perforant path fibers respectively. Colchicine was dissolved in the perfusate in concentrations of 0.01 to 10mM.

In CA_1, low concentrations (0.03-0.3mM) of colchicine increased the number of population spikes (maximum number 10; median 6) without significantly changing their amplitude or those of population epsp's. In 2 slices epileptiform burst discharges were triggered with stimuli delivered at 1 Hz. With concentrations of colchicine $\geq$ 1mM, multiple population spikes persisted, but the amplitude of population spikes and population epsp's decreased in parallel. Upon rinsing with colchicine-free perfusate both evoked responses normalized. The effects of colchicine on the dentate gyrus were similar although the increase in number of population spikes was less impressive (maximal number 4, median 2) and no epileptiform events were noted.

These results indicate that colchicine can have several effects on synaptic transmission, depending on the amount of the drug present, both at pyramidal cells in the hippocampus and at granule cells in the dentate gyrus. Lower concentrations of the drug are "excitatory" while higher concentrations block evoked responses.

330.16 THE EFFECTS OF NA-PENTOBARBITAL AND URETHANE ON SYNAPTIC ACTIVITY IN THE DENTATE GYRUS OF THE POSTNATAL RAT. D.A. Wilson* and R.J. Racine. Dept. Psychology, McMaster University, Hamilton, Canada. (spon: H. Weingarten).

The immature CNS has been shown to be more sensitive to barbiturates than the mature CNS (Bianchine & Ferguson, Proc Soc Exp Biol & Med, 1967,124,1077). However, the examination of barbiturate sensitivity in the past appears to have been limited to measures such as duration of induced narcosis and lethal dose. The present study was an examination of paired-pulse facilitation/depression in the dentate gyrus of postnatal rats (PN14-adult), anesthetized with either pentobarbital or urethane, or unanesthetized.

Stimulation of the perforant path produced a positive EPSP, recorded in the dentate hilus, in all age groups. A negative population spike was superimposed on the EPSP at higher stimulation intensities. Paired pulse testing involved applying a single conditioning pulse (producing 80-100% maximal spike) followed at 20-5000msec by an equal intensity test pulse. The area of the spike was used as response measure. Percent facilitation/depression was calculated as (test response/conditioning response) x 100.

In both urethane anesthetized and unanesthetized animals, short inter-pulse intervals (20msec IPI) produced a marked depression of the test spike. This depression is mainly due to recurrent inhibition (Andersen, et al, Acta Physiol Scand, 1966,66,448). Following this initial depression, a pronounced facilitation was apparent which then decayed by 300-500msec.

Pentobarbital increased the duration of the initial depression in all age groups, as expected. Barbiturates potentiate GABA-mediated IPSP's (Nicoll, et al, Nature, 1975, 258,625). However, the magnitude and duration of this depression increased markedly with decrease in age. Whereas a net facilitation was present at longer IPI's in pentobarbital anesthetized adult animals, facilitation in immature animals was completely masked by a long-lasting (1000-3000msec) depression.

These results suggest that the sensitivity of the GABA inhibitory system to barbiturates is enhanced in the immature CNS.

330.17 DITHIZONE INCREASES GRANULE CELL RESPONSES EVOKED BY THE PERFORANT PATHWAY IN THE HIPPOCAMPAL SLICE PREPARATION. H.J. Doller* and I.L. Crawford, (SPON: M.R. Vasko) VAMC, Dallas, Tx and UTHSCD, Dallas, Tx.

Two regions of the hippocampal formation, the CA3 mossy fiber terminals and a region just below the granule cell (GC) bodies, are both prominently stained after parenteral dithizone. The drug may be chelating zinc, but the function of the metal is unknown. Concomitant with CNS staining, dithizone (100 mg/kg, i.p.) has been reported to specifically block CA3 mossy fiber transmission. The chelator's effects on GC electrophysiology have not been reported.

The action of dithizone on responses evoked in GC by perforant pathway stimulation was investigated in the guinea pig hippocampal slice preparation. GC responses were recorded with glass electrodes (2 to 5 Megohms). The perforant fibers were stimulated at a rate which did not cause frequency potentiation (1/10 sec). Responses were quantified by determining the area of the population spikes. 1 and 5 mg/l dithizone resulted in no or very slight staining of the mossy fibers. 10 to 50 mg/l stained with a similar pattern and intensity as seen in whole animals (100 mg/kg). At 50 mg/l stain was also seen in the striatum radiatum of CA1. Low doses (1 to 5 mg/l) increased the area of the population spike by 25% to 40%; high doses (10 to 50 mg/l) decreased or completely inhibited the spike.

The initial hypothesis was that low doses of dithizone would have no effect on evoked GC responses since perforant terminals do not stain and the chromagen seen in the GC region is generally thought to be within boutons of GC collaterals which terminate on inhibitory basket cells. One possible mechanism for the unexpected potentiation may be that some GC are spontaneously active. This activity could tonicly excite basket cells, which in turn could tonicly inhibit a large population of GC. Low dithizone doses might reduce the tonic inhibition and thereby facilitate the GC population spike. Another possibilty is that dithizone has a direct effect on perforant pathway terminals or the GC. Interpretation of the inhibitory effects seen with high dithizone concentration will require determination of plasma dithizone levels following 100 ml/kg, i.p. injections.

Supported by VA Merit Review Research Programs.

330.18 ANTAGONISM OF MEDIAL PERFORANT PATH RESPONSES BY BACLOFEN, ADENOSINE AND KYNURENIC ACID: DIFFERENTIATION OF PRE- AND POSTSYNAPTIC ANTAGONISM. Eric W. Harris and Carl W. Cotman, Department of Psychobiology, University of California, Irvine, CA 92717.

Using the in vitro hipocampal slice preparation, we have examined the effects of several antagonists of perforant path synaptic transmission with respect to their effects on paired-pulse habituation. Two new antagonists of medial perforant path responses are also described, and these are found to have differential effects on the response to paired stimuli, consistent with either presynaptic or postsynaptic antagonism.

Pairs of stimuli separated by 60 or 160 msec were delivered to the medial perforant path every 20 seconds, and the waveforms were compared online using a microcomputer. Under normal conditions, the medial perforant path shows a pronounced decrement of the second response ("habituation"). Addition of 2-5 uM baclofen causes a 40-80% decrease in the first evoked response, but the response to the second stimulus becomes larger than the first. Thus, under baclofen inhibition, paired-pulse habituation changes to paired-pulse potentiation. This effect does not result from contamination by lateral perforant path responses (which are baclofen insensitive and show paired-pulse potentiation), because identical results are obtained when 20uM +APB, which reduces lateral perforant path responses, is added before or during baclofen treatment. A similar change in the response to paired stimuli is caused if responses are decreased by reducing extracellular Ca^{++}, or by presenting several pairs pairs of stimuli at a shorter interpair interval. Adenosine is also a potent blocker of medial perforant path responses at 10uM and, like baclofen, changes habituation into potentiation. In contrast, addition of 250-800 uM kynurenic acid reduces the medial perforant path response by 40-80% but does not change the relationship between the first and second responses.

These results support the idea that baclofen and adenosine are presynaptic antagonists, and suggest that habituation, like paired-pulse potentiation, is a presynaptic process; the increase in, or unmasking of, potentiation is then an expected consequence of reducing presynaptic release. Kynurenic acid, which appears to be a postsynaptic receptor blocker, reduces the response postsynaptically, and so does not interact with the processes underlying paired-pulse habituation.

Supported by NIH NS0857 and Postdoctoral NS06480 (EWH)

330.19 HIPPOCAMPAL IPSPS ARE PROLONGED BY DRUGS WHICH INHIBIT GABA-UPTAKE. Stephen J. Korn and Raymond Dingledine. Dept. of Pharmacology, Univ. of North Carolina, Chapel Hill, NC 27514.

The processes that regulate the timecourse of gamma-amino butyric acid (GABA)-mediated ipsps are not known. Following the discovery of GABA-uptake processes in several neuronal and glial tissue preparations, it was proposed that GABAergic transmission was temporally limited by the active transport of GABA from the extracellular space into neurons or glia. However, physiological studies of GABAergic transmission in vivo have failed to provide support for this hypothesis. We are using the in vitro hippocampal slice preparation to determine the effect of inhibition of GABA uptake on the timecourse of GABA-mediated ipsps. Intracellular recordings were made from pyramidal cells in region CA1 of rat hippocampus. Iontophoresis of GABA (1M, pH 4.65 or 0.5M, pH 4) near the impaled cell resulted in a conductance increase accompanied by a hyperpolarization (somatic application) or a depolarization (dendritic application). GABA-mediated ipsps were evoked by excitation of inhibitory interneurons via electrical stimulation of the pyramidal cell axons (antidromic) or Schaffer collaterals (orthodromic). In order to eliminate regenerative sodium conductances, the recording electrodes were filled with the quaternary lidocaine derivative, QX-314 (50-200mM). Hyperpolarizing current pulses (.25-.50nA) were injected into the cell through the recording electrode to measure input resistance. The GABA uptake blockers, nipecotic acid, cis-4-OH nipecotic acid (CIS) and L-2,4 diaminobutyric acid (all at 1mM), prolonged and often potentiated responses to iontophoresed GABA, while producing little or no direct effect on the resting potential or input resistance. Perfusion of the slices with CIS (0.3-1mM), an uptake blocker which acts selectively at glial uptake sites, reversibly prolonged both antidromic and orthodromic ipsps. Perfusion with 1mM nipecotic acid prolonged orthodromic ipsps; its effect on antidromic ipsps is currently unclear. The peak amplitudes of the ipsps were usually reduced in the presence of uptake blockers. However, ipsps were prolonged by these uptake blockers at all stimulus intensities and regardless of the magnitude of the ipsp. Further, penicillin, which reduces ipsps via a postsynaptic mechanism, did not affect the timecourse of ipsps. The effects of CIS and nipecotic acid on ipsps were usually rather small, and differed qualitatively from that of pentobarbital (100 µM), which greatly prolonged and potentiated both orthodromic and antidromic ipsps. These results suggest that reuptake of GABA into neurons or glia may play a role in the termination of transmitter action at GABAergic synapses in the hippocampus. We thank Dr. P. Krogsgaard-Larsen for supplying several GABA agonists and uptake bockers, and Dr. Bertil Takman for his gift of QX-314. Supported by NS-17771.

330.20 PHARMACOLOGICAL ANTAGONISM OF CLIMBING FIBER-, PARALLEL FIBER-, AND ACIDIC AMINO ACID-INDUCED EXCITATION OF FROG CEREBELLAR PURKINJE CELLS. S.L. Cochran. Brain Research Institute, Univ. of Zürich, August-Forel-Strasse 1, CH-8029 Zürich, Switzerland.

Aspartate and glutamate are thought to be the neurotransmitters released from the climbing fiber (CF) and parallel fibers (PF's) respectively, but their 'identity of action' with the natural transmitter substance is not established. According to this criterion, pharmacological blockade of the action of the synaptic transmitter should also block the action of the exogenously applied substance. In order to test for such similarity, experiments were performed upon isolated, intact frog cerebella. Acidic amino acid antagonists were in most cases bath-applied, and Purkinje cell (PC) excitation, selectively induced by PF's, by the CF, and by iontophoretically-applied amino acids (usually aspartate), was concomitantly monitored with extracellular recordings. Glutamic acid diethyl ester and α-aminoadipic acid have no effect upon PF-, CF-, or aspartate-induced PC excitation. γ-D-glutamylglycine, 2-amino-4-phosphonobutyric acid, 2-amino-5-phosphonovaleric acid, cis-2,3-piperidine dicarboxylic acid, and baclofen all reversibly block spike potentials from PF stimulation, reduce iontophoretic aspartate-induced excitation (baclofen weakly), and do not effect the CF-evoked response, the antidromically-evoked action potential, or the spontaneous firing rate of the PC. None of these substances, even when applied at high concentrations (5 mM) for long periods of time (20 min), blocked the CF response. On the other hand, a 1 min application of 5 mM kynurenic acid (KENYA) noticeably reduces the CF-evoked burst. KENYA also blocks PF-induced excitation and reduces that of the acidic amino acids. Glutamate and quisqualic acid are much less reduced in their action than are N-methyl-D-aspartic acid, quinolinic acid, kainic acid, or aspartate, whose actions are nearly abolished by KENYA. γ-D-glutamylaminomethylsulfonic acid (GAMS) is similar in its action to KENYA. Both GAMS and KENYA do not influence the spontaneous firing rates of PC's, their antidromic activation, or carbachol-induced excitation and GABA-induced inhibition of PC's, suggesting that these substances act as sub-synaptic antagonists. The lack of specificity of KENYA and GAMS antagonism (or lack of specificity of the sub-synaptic receptor(s) itself) hinders a distinction between these acidic amino acids as to which, if any, are the actual transmitter substances. Streptomycin blocks both CF- and PF-induced excitation, but increases the response to glutamate and aspartate. These studies do not clarify the role of aspartate or glutamate in the cerebellum.

330.21 ETHOSUXIMIDE AND TETRAMETHYLSUCCINIMIDE EFFECTS ON CULTURED CORTICAL NEURONS. Deborah M. Barnes and Marc A. Dichter, Dept. of Neuroscience, The Children's Hospital, Boston, MA 02115.

The physiological effects of the anticonvulsant, ethosuximide (ESM) and the convulsant tetramethylsuccinimide (TMSM) were evaluated using rat cerebral cortical neurons grown in primary culture. ESM (Zarontin) has widespread clinical use in the treatment of absence seizures, and we examined the possibility that its cellular mechanism of action was to enhance the effects of either GABA- or glycine-mediated inhibition. No concentration of ESM tested (10 uM - 1 mM) increased neuronal responses to exogenously applied GABA or glycine. Surprisingly, ESM (> 100 uM) decreased both GABA- and glycine-mediated changes in membrane conductance. ESM (1 mM) did not reduce the GABA blocking effects of picrotoxinin. ESM did not produce any consistent changes in resting membrane potential, input resistance, spontaneous PSPs, or action potentials.

TMSM produces convulsions and epileptiform *discharges in animals (Klunk et al., 1982, Molec.* Pharmacol., 22:444-450) and causes spontaneous paroxysmal discharges in incubated hippocampal slices (Klunk, Covey, and Ferrendelli, personal communication). TMSM (100 uM - 1 mM) reduced the amplitude of spontaneously occurring GABAergic IPSPs in cultured cortical neurons and decreased neuronal responses to exogenously applied GABA. Equimolar concentrations of ESM were unable to block these TMSM-mediated effects. TMSM did not modify glycine-induced conductance increases.

The convulsant actions of TMSM appear to involve an antagonism of GABA-mediated inhibition. The anticonvulsant properties of ESM are not due to an enhancement of either GABA- or glycine-mediated effects.

Supported by NIH grants NS06869, NS15362, and the CHMC Mental Retardation Core Grant HD06276.

330.22 SELECTIVE AND REVERSIBLE BLOCKADE OF THE SLOW-IPSP BY GALLAMINE IN RABBIT SUPERIOR CERVICAL GANGLION. C. A. YAROSH* and JOHN H. ASHE. Dept. of Psychology, Univ. of Calif., Riverside, CA 92521.

In superior cervical ganglion (SCG), elicitation of the S-IPSP and S-EPSP requires the interaction of ACh with muscarinic cholinergic receptors. It has been suggested that these postsynaptic responses involve activation of pharmacologically distinct muscarinic receptors; a subpopulation designated M-1, for production of the S-EPSP, and a subpopulation, termed M-2, for production of the S-IPSP(Gardier et.al., JPET, 204:46, 1978; Brown et.al., Brit. J. Pharmacol. 71:362, 1980).

In the cat SCG, there is evidence that the generation of the S-IPSP can be antagonized by the neuromuscular blocking agent, gallamine(Gardier et.al., JPET, 204:46, 1978). The experiments reported here characterize this action of gallamine on postsynaptic potentials of the rabbit SCG using extracellular recording techniques. Concentration-response analyses are presented that illustrate the actions of gallamine over a range of stimulus train durations. A concentration of 28uM suppresses the amplitude of the S-IPSP by 80-100%. This profound blockade of the S-IPSP can be obtained with little or no suppression of the S-EPSP, or the atropine-sensitive asynchronous afterdischarge *recorded in the postganglionic nerve. Orthodromic stimulation* with trains of durations of 5sec or less does not produce any obvious increase in the amplitude of the S-EPSP even though the overlapping S-IPSP is greatly suppressed. Stimulation with trains of longer duration (e.g. 10sec) can, however, result in some increase in S-EPSP amplitude.

Gallamine blockade of the S-IPSP is rapidly reversed by incubation in gallamine-free medium. Tetanic stimulation of the preganglionic nerve, which presumably increases the release of ACh to subsequent test stimuli, also produces an almost complete recovery of S-IPSP amplitude.

The action of gallamine appears to be specific for those muscarinic receptors associated with the S-IPSP. The S-EPSP is unaffected. Likewise, there is no effect on the amplitudes of the nicotinic F-EPSP or the noncholinergic SS-EPSP.

(Supported by NSF Grant PRM-8200575 and PSH BRSG-RR07010-17).

330.23 FACILITATION OF REPETITIVE SYNAPTIC ACTIVITY IN POSTGANGLIONIC NEURONS BY ADENOSINE AND NORADRENALIN. Barbara K. Henon and Donald A. McAfee. Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

Adenosine and noradrenalin are generally considered to be inhibitors of neuronal activity. However, in the superior cervical ganglion they have the interesting property of inhibiting nicotinic fast EPSPs but facilitating the transmission of repetive activity (Henon and McAfee, Soc. Neurosci.Abst. 1982). In order to determine whether presynaptic and/or postsynaptic effects of these neuromodulators are responsible for their facilitative actions we have examined the frequency response of individual postganglionic neurons by two different stimulus paradigms. 1. Non synaptic effects of the agonists were studied by stimulating postganglionic neurons directly through the micro-electrode with a train of 15 stimuli at frequencies of 5-30 Hz. The number of action potentials evoked/train decreased with increasing frequency. Noradrenalin (10 μM) increased the number of action potentials to 153 + 14% of control in 5 out of 6 cells. The adenosine analog, 2-chloroadenosine (10 μM) increased firing to 144 + 19% of control (n = 4). Low external Ca^{2+} (1.2 mM) increased firing to 156 + 28% (n = 3). High Ca^{2+} (3.4 mM) which augments the hyperpolarizing afterpotential (HAP) depressed firing by 59% (n = 2). There is an inverse correlation between the amplitude or duration of the HAP and the percentage of firing. 2. The synaptic effects of the neuromodulators were studied by stimulating the preganglionic nerve with a train of 15 stimuli. At low frequencies, residual EPSPs usually produce afterdepolarizations. At higher frequencies (15-30 Hz) the action potentials are attenuated and the EPSPs summate to produce a large depolarization (8-20 mV). Noradrenalin, 2-chloroadenosine, low Ca^{2+} and curare reversibly reduced the afterdepolarizations as well as the large depolarizations. Noradrenalin increased the ratio of action potentials to EPSPs by 279 + 47% of control (n = 6). 2-chloroadenosine increased firing to 132 + 10% (n = 5) while 1.2 mM Ca^{2+} increased firing to 408 + 63% in 7 cells. High Ca^{2+} (3.4 mM) depressed firing by 68.3 + 1% (n = 3). The acetylcholine (ACh) antagonist, curare (25 μM), had an effect similar to adenosine and noradrenalin during synaptic stimulation, increasing firing to 283 + 36% of control in 7 cells. However, unlike the modulators, curare had no effect on the HAP nor on the response to direct stimulation (n = 6).

Inhibition of the HAP by the neuromodulators increases excitability by a postsynaptic action. In addition, presynaptic inhibition of ACh release by the neuromodulators (or postsynaptic antagonism by curare) may facilitate transmission by reducing the action of excess ACh which causes postsynaptic shunting during repetitive synaptic activity. (Supported by grants NSF BNS 81-12414 and NIH NS-18996).

330.24 CHARACTERIZATION OF A SNAKE VENOM NEUROTOXIN WHICH BLOCKS NICOTINIC TRANSMISSION IN AUTONOMIC GANGLIA. R.H. Loring, V.A. Chiappinelli, R.E. Zigmond and J.B.Cohen, Dept. of Pharmacology, Harvard Med.School, Boston, MA 02115.

In order to determine whether Bungarus multicinctus venom contains a neurotoxin(s) which might be useful in characterizing ganglionic nicotinic receptors, venom was fractionated by ion exchange chromatography and the various fractions were assayed for their ability to block synaptic transmission through the chick ciliary ganglion. α-Bungarotoxin purified from this venom failed to block transmission at 50 μg/ml. A second neurotoxin, which we designate toxin F, blocked transmission at 1-3 μg/ml. Toxin F also blocked carbachol-induced ganglionic depolarizations, which suggests that this toxin blocks transmission via a postsynaptic mechanism. In addition, toxin F blocked synaptic transmission through the rat superior cervical ganglion at 10-30 μg/ml. Toxin F was clearly distinguishable from α-bungarotoxin on the basis of its molecular weight, estimated by SDS gel electrophoresis, (∿ 6,500 vs. ∿ 8,000) and its isoelectric point (pI ∿ 8.7 vs. pI > 9.2). Unlike β-bungarotoxin, a presynaptically acting neurotoxin, toxin F contained no detectable phospholipase activity. Preliminary results from amino acid analysis indicate that toxin F has only one tyrosine residue and no methionine. The amount of recovered toxin F represented 0.2% of the crude venom by weight.

Binding assays revealed that ^{125}I-labeled toxin F bound to two sites in the chick ciliary ganglion: one site that was recognized by α-bungarotoxin and toxin F and another site that was recognized solely by toxin F. Of the two sites, the second site is more likely to represent the site at which toxin F exerts its effect, since toxin F blocks transmission even in the presence of excess α-bungarotoxin. To test whether this second site is also recognized by other nicotinic agents, the binding of ^{125}I-toxin F was studied in the presence of high concentrations of carbachol and d-tubocurarine. Neither drug inhibited the binding of toxin F to the second site.

The present physiological data demonstrates that toxin F blocks synaptic transmission in the chick ciliary ganglion and in the rat superior cervical ganglion. While toxin F's action in the ciliary ganglion appears to be postsynaptic, the mechanism by which transmission is blocked remains to be determined.

Supported by USPHS NS12651, NS12408, NS07009, and MH00162.

330.25 EVIDENCE FOR A SEROTONINERGIC TRANSMISSION IN GUINEA PIG CELIAC NEURONS. R. C. Ma*, M. Kiraly*, T. H. Chiu*, M. A. Simmons and N. J. Dun. Dept. Pharmacol. Loyola Univ. Med. Ctr., Maywood, IL 60153.

Synaptic transmission in neurons of the guinea pig isolated left celiac ganglia was investigated by means of intracellular recording techniques. Stimulation of the left greater splanchnic nerves evoked a fast excitatory postsynaptic potential (epsp) that could be reversibly suppressed by nicotinic antagonists. Repetitive nerve stimulation (10-20 Hz, 1-2 sec) elicited, in addition to the initial fast epsp's, a slow depolarization lasting for min. The slow depolarization was not affected by nicotinic and muscarinic antagonists, but was reversibly abolished in a low Ca/high Mg solution indicating that the response was postsynaptic, and was probably mediated by a noncholinergic transmitter(s). The mean amplitude and duration of the noncholinergic epsp were 5.1±2.7 mV and 158±123 sec, respectively (n=325, S.D.). The noncholinergic epsp was accompanied by an increase of membrane resistance in a large majority of the cells tested; the increase could still be observed when the membrane potential was restored to the resting level by hyperpolarizing currents. Membrane hyperpolarization generally caused an increase of the amplitude of noncholinergic epsp; a decrease was noticed in only a very few cells. In the latter neurons a reversal potential was not observed when the membrane was hyperpolarized to a level more negative than the estimated potassium equilibrium potential. In about 50% of the celiac neurons tested application of serotonin (5-HT, 1-10 μM) caused a slow membrane depolarization with electrophysiological characteristics very similar to that evoked by nerve stimulation. Furthermore, the noncholinergic epsp elicited in 5-HT sensitive neurons was completely abolished by prolonged application of 5-HT. Pharmacological studies showed that the depolarization induced by either nerve stimulation or 5-HT was markedly enhanced by fluoxetine (30-50 μM), a 5-HT reuptake blocker, and suppressed by cyproheptadine (50 μM), a known 5-HT receptor antagonist. Additionally, superfusing the ganglia with the 5-HT precursor, L-tryptophan (50 μM), augmented the noncholinergic epsp while the membrane depolarization induced by 5-HT was not significantly increased. Lastly, dense but unevenly distributed nerve fibers exhibiting 5-HT immunoreactivity could be demonstrated in celiac ganglia pretreated with L-tryptophan (50 μM) by means of immunohistofluorescent techniques. The 5-HT immunoreactive fibers were observed to run parallel and come into close proximity with many ganglionic neurons. Immunoreactivity was not observed following the incubation of the ganglia with anti-sera pre-absorbed with excess 5-HT. Collectively, our results support the notion that 5-HT is the transmitter responsible for the generation of a slow noncholinergic epsp in some celiac neurons. (Supported in part by NS15848).

330.26 BACLOFEN: PHYSIOLOGICAL EVIDENCE FOR A PRESYNAPTIC SITE OF ACTION IN SPINAL CORD CULTURES. R.Y.K. Pun and G.L. Westbrook. Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, Md. 20205

Baclofen is a lipophilic analogue of GABA which is used clinically in the treatment of spasticity. However, at therapeutic concentrations baclofen does not mimic the postsynaptic increase in chloride conductance characteristic of GABA. Thus baclofen's mechanism of action has been unclear. Recent neurochemical and ^3H-baclofen binding data have suggested a presynaptic action of baclofen at a novel $GABA_B$ receptor(Bowery, TIPS 3: 399, 1982). We present physiological evidence that baclofen reduces monosynaptic excitatory postsynaptic potentials between cultured spinal cord neurons(SC-SC EPSPs) by reducing quantal number; and that this presynaptic action has pharmacological properties similar to a baclofen-induced reduction of somatic calcium current in voltage clamped spinal cord neurons.

Primary dissociated cultures of embryonic mouse spinal cord were maintained for 3-6 weeks before physiological study. Monosynaptic EPSPs were identified on the basis of a short fixed latency to repetitive stimulation. Quantal analysis was performed using the coefficient of variation(CV) method. Baclofen(10^{-4}-10^{-7} M) was applied by miniperfusion to assess the effect of the the drug on transmitter release from the presynaptic terminals [quantal number = $1/(CV)^2$]. As in vivo, baclofen produced a marked reduction in EPSP amplitude(n=33). The decrease in EPSP amplitude was entirely attributable to a decrease in quantal number (Linear regression slope = 1.0, r=.95). Dose response curves on 5 EPSPs gave an ED_{50} of approximately 5 μM for the effect on on both EPSP amplitude and quantal number. The (-)stereoisomer was much more potent than the (+)stereoisomer(n=7).

Baclofen has been reported to reduce calcium spikes in cultured dorsal root ganglion cells(Dunlap, Br.J.Pharmacol. 74: 579, 1981). To assess baclofen's action on somatic calcium currents, SC neurons were voltage clamped with two CsCl-filled microelectrodes in a bath containing 1 μM TTX, 25 mM TEA, 5 mM Ca^{++}, and 1 mM Mg^{++}. Slow inward currents were evoked by 30 msec step pulses to 0 mV from a holding potential of 50-60 mV. (-)Baclofen (10^{-4} M) reversibly reduced the peak inward current(%reduction ± sem = 28 ± 2.4%, n=14). This effect was stereospecific(n=4) and was not antagonized by 5 μM bicuculline(n=3). However, the reduction in somatic calcium current was less than the reduction in quantal number for the equivalent drug concentration.

We conclude that baclofen acts presynaptically on cultured SC-SC EPSPs to reduce the release of transmitter. The reduction of somatic calcium current by baclofen suggests a possible mechanism for its action at the presynaptic terminals.

330.27 MONOSYNAPTIC EXCITATORY POSTSYNAPTIC POTENTIALS AND RESPONSES TO PUTATIVE AMINO ACID NEUROTRANSMITTERS IN SPINAL CORD CULTURES: A VOLTAGE CLAMP STUDY. P.G. NELSON, R.Y.K. PUN & G.L. WESTBROOK Lab. Dev. Neurobiol., N.I.C.H.D, N.I.H., Bethesda, MD, 20205.

The reversal potential of monosynaptic excitatory postsynaptic potentials (EPSPs) between spinal cord (SC) cells in cultures was determined to be +2.7 ± 2.5 mV (mean ± s.e.m.; Pun, Westbrook & Nelson, Neurosci. Abs., 8: 492, 1982). To further characterize the nature of this synapse, we studied the EPSPs between SC neurons under voltage clamped condition.

Dissociated SC cells from fetal mice were used for experiments after 3 - 6 weeks in culture. Criteria for selecting EPSPs are as described (see Pun & Westbrook, these abstracts). Cells were voltage clamped with two electrodes. A patch-type pipette (see Hamill et al., Pflugers Arch., 391: 85, 1981) containing 10 mM Cs in EGTA-buffered solution was used as the current passing electrode. Membrane potential was held at -40mV. 10mV voltage steps were made to construct I-V plots for the synaptic current. On several cells, synaptic currents evoked by recurrent collateral (Nelson et al., J. Neurophysiol., In press, 1983) were observed following a short depolarizing step to the cell under clamp. Results from the two groups of studies were similar and therefore treated together.

On termination of a study, the presynaptic cell was injected with the fluorescent dye lucifer yellow CH (Sigma) to determine the spatial distribution of the terminals on the postsynaptic cell. Most terminals were found to have a proximal locality.

Peak current was reached in about 1 - 1.5 msec and was linearly related to the membrane voltage. The rate of decay of the current had no obvious voltage dependence between -20 and -50mV. Reversal potential of the currents was approximately 0mV.

Responses on SC cells to the putative neurotransmitter glutamate (GLU) and aspartate (ASP) were examined in the presence of 1 μM tetrodotoxin. Conventional micropipettes filled with CsCl were used for clamping the neurons. Drugs were applied with fixed ejecting currents at regular intervals to ensure a constant delivery of drug during each ejection period. Both drugs induced an inward current at hyperpolarising holding potentials. Reversal potential for both amino acids was near 0mV. The response to ASP was highly voltage dependent between -30 and -10mV (see MacDonald, Porietis & Wojtowicz, Brain Res., 237: 248, 1982). This voltage dependency was present but less evident on responses to GLU, and was not observed for the synaptic currents in the cell pairs we have studied to date. A more detailed analysis of the kinetics of the currents coupled with pharmacological studies may help to resolve whether GLU and ASP are transmitters mediating the EPSPs in SC cultures.

330.28 ENKEPHALIN SELECTIVELY MODULATES SYNAPTIC TRANSMISSION IN MOUSE SPINAL CORD CELL CULTURES, M. Jia* and P.G. Nelson (SPON: B. Schrier), Lab. of Dev. Neurobiology, IRP, NICHD, NIH, Bethesda, MD, 20205.

Monosynaptic excitatory post-synaptic potentials (EPSPs) evoked in spinal cord (SC) neurons by stimulation of dorsal root ganglion (DRG) neurons in cell cultures were reduced by perfusion application of the opiate peptide, met-enkephalin (2-4μM). In about one-half of cases examined, EPSPs evoked by stimulation of spinal cord cells were also reduced by met-enkephalin. The effects were antagonized by concommitant perfusion with naloxone (1 μM) and reversed when perfusion with met-enkephalin was stopped. Statistical analysis of synaptic responses indicated that the reduction of EPSP amplitude was due, at least to a major extent, to a decrease in presynaptic transmitter release. This effect is similar to that seen with the opiate, etorphine (Macdonald, R.L. and Nelson, P.G., Science 199: 1449-1451, 1978). Opiate peptides have been shown to reduce the voltage sensitive calcium conductance in chick and mouse DRG neurons (Mudge, A.W. et al., PNAS 76: 526-530, 1979; Werz, M.A. and Macdonald, R.L., Brain Res. 239: 315-318, 1982). The fact that enkephalin affected transmitter output in only half of spinal cord neurons tested argues against a direct effect of enkephalin on some components of the transmitter release apparatus such as calcium channels and for a selective distribution of an enkephalin receptor system on different types of neurons.

330.29 INCORPORATION OF FUNCTIONAL DOPAMINE RECEPTORS INTO PLANAR LIPID BILAYER MEMBRANES. S.W. Michnick* & P. Seeman (SPON: J.W. Scott). Dept. Pharmacol., University of Toronto, Toronto M5S 1A8.

The incoporation of dopamine receptors into planar lipid bilayers could serve as a model for the electro-physiological function of dopamine receptors provided that in this system the receptor can elicit electrical responses. We here report that we were able to incorporate dopamine receptors linked to electrical conductance channels that were activated by the dopamine receptor agonists apomorphine and dihydroxyaminotetralin (ADTN), and that these conductances were blocked by the potent dopamine antagonist (+)-butaclamol.

A rat striatal homogenate was suspended in 15 mM 3-(N-morpholino) propanesulfonic acid (MOPS), and 50 mM sucrose at pH 7.4. Membrane vesicles were prepared from this suspension by passing the homogenate through a 27 gauge needle ten times. These vesicles were incorporated into essentially solvent-free phosphatidylethanolamine bilayer membranes. The membrane was generated on a teflon aperture of 2 mm^2 area separating two compartments that both contained a solution of 20 mM KCl, 20 mM NaCl, and 2 mM $CaCl_2$ at pH 7.4. Electrical current and capacitance of the membrane were monitored with a voltage clamp circuit. The bilayer membrane (containing fused striatal vesicles) were exposed to the dopamine agonists apomorphine and ADTN at increasing concentrations (0-300 nM) until a change in the conductance of the membrane was elicited. The membranes were then exposed to the potent dopamine receptor antagonist (+)-butaclamol or its inactive enantiomer (100-500 nM).

In 7 independent experiments apomorphine caused an increase in the conductance of the membrane on the order of 10^{-9} $ohms^{-1}$ x cm^{-2} at final concentrations of 10-300 nM. (+)-Butaclamol completely reversed the apomorphine-induced conductance changes whereas (-)-butaclamol had no effect. Similar results were obtained using ADTN.

The low concentrations of dopamine receptor agonists used in these experiments to elicit responses and the stereoselective actions of butaclamol in blocking these responses suggests specific activation of incorporated dopamine receptors linked to electrical conductance channels (see Reference 1).

1. R.B. Murphy and V. Vodyanoy. Neurosci. Abstr. 8: 832 (1982).

331.1 NICOTINE MIMICS TETRAHYDROCANNABINOL AND LEVONANTRADOL POTENTIATION OF RESERPINE-INDUCED HYPOKINESIA. S. P. Montgomery* and D. E. Moss. Department of Psychology, University of Texas at El Paso, El Paso, Texas 79968.

Moss, McMaster and Rogers (Pharmac. Biochem. Behav. 15, 779-783, 1981) have shown that delta-9-tetrahydrocannabinol (THC) produces a 20 fold potentiation of reserpine (RES)-induced hypokinesia in rats as measured by the bar test. A similar THC potentiation of haloperidol-induced hypokinesia has also been observed (Moss, unpublished). These results are useful for the elucidation of a neuropharmacological mechanism of action for THC because RES-induced hypokinesia is a well studied animal model of Parkinson's disease. Therefore, this model has three important advantages: 1) the extrapyramidal neuroanatomical substrate for RES hypokinesia is relatively well known, 2) the neurotransmitters interacting in the extrapyramidal system have been at least partially identified, and 3) the resulting behaviors are exquisitely sensitive to THC and are easily and reliably measured.

As an extension of the results obtained with THC, levonantradol (LEVO), a synthetic analog of THC, was tested in this model and was found to be very powerful. Whereas the average bar time for RES alone was 40 sec and the time for THC/RES was 15 min, the time for LEVO/RES was 30 min (the maximum time tested). Actually, some LEVO/RES animals stood more than 1 hr and one exceeded 2 hrs.

In view of the finding that THC/RES hypokinesia was not blocked by L-DOPA but was dramatically reduced by ethopropazine (Parsidol, an anticholinergic antiparkinsonian drug), further studies with cholinergic drugs were conducted. Surprisingly, THC/RES hypokinesia was only slightly enhanced by physostigmine and relatively unaffected by scopolamine (Moss et al., 1981). We have recently discovered, however, that nicotine (1 mg/kg IP) potentiates RES-induced hypokinesia in the same dramatic fashion as THC (average bar time 12 min). It should also be noted that THC, LEVO and nicotine do not have a hypokinetic effect without RES treatment.

It appears that THC's action in the extrapyramidal system is a minor subtle effect that is masked by the function of the main dopamine (DA) system. When the DA system is blocked, however, (e.g., with RES or haloperidol), the normally occult effect(s) of THC, LEVO and nicotine become observable. Our current working hypothesis is, therefore, that THC affects extrapyramidal function, either directly or indirectly, through a cholinergic mechanism that is possibly nicotinic. We are currently making intracranial injections of various drugs in order to specifically identify a necessary site and mechanism of action.

Acknowledgments: LEVO was a kind gift from Pfizer Pharmaceutical and THC was generously supplied by NIDA. Supported in part by MBRS Grant RR08012.

331.2 EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL (THC) ON ENDOGENOUS OPIOIDS AND OPIOID RECEPTORS. A.S. Bloom, T.M. Dieringer*, J.K. Greathouse* and J.K. Kobs*. Dept. of Pharmacology and Toxicology, Med. Col. of Wisconsin, Milwaukee, WI 53226.

We have previously reported that the hypothermic action of THC was partially antagonized by naloxone and that there was a limited cross-tolerance between THC and morphine (Psychopharmacology 57: 243-248, 1978). These observations suggested that THC and other behaviorally active cannabinoids could interact with endogenous opioid systems. The following studies were performed in order to further examine the effects of THC on endogenous opioid systems.

Male ICR mice were injected twice daily with increasing doses (25 to 200 mg/kg ip) of THC or Emulphor vehicle for six days. In mice receiving 6 days of vehicle treatment, an icv injection of 10 µg of β-endorphin (END) produced a 2.80 ± .45°C decrease in body temperature. The same dose of END in THC treated animals produced a 0.78° ± .18°C decrease. Under the same conditions, a 2 mg/kg iv dose of THC produced a 2.23 ± .4°C decrease in the vehicle treated mice and a 0.41 ± .36°C decrease in the THC treated animals. This study indicates that mice who are tolerant to the hypothermic action of THC are also cross-tolerant to the same action of END.

The effects of acute and chronic treatment with THC on brain levels of END, met-enkephalin (MET) and leu-enkephalin (LEU) were studied. Opioid levels were measured using single antibody RIA methods. Neither acute treatment (0.1 to 8 mg/kg) nor chronic treatment (6 days) with THC significantly altered the levels of END in whole brain or hypothalamus, or MET and LEU in the striatum.

The effects of THC on the binding of ^{3}H-naloxone (NAL) and ^{3}H-D-Ala^{2}-D-Leu^{5}-enkephalin (DADL) to mouse brain membranes were also examined. THC, in vitro produced a concentration related inhibition of NAL binding. The IC_{50} for THC using 1 nM NAL was 19.8 µM. Saturation analysis of NAL binding in the presence of 20 µM THC or vehicle indicated that the decrease in binding was due to a increase in the apparent K_d for NAL without a significant change in the Bmax. The K_d in the presence of vehicle was 0.94 nM and in the presence of THC was 3.1 nM. In contrast to the effect on NAL binding, DADL binding was unaffected by THC concentrations as high as 40 µM. This suggests that THC, in vitro, can have a selective effect on mu opiate receptors without significantly altering binding to delta receptors. (Supported by USPHS Grant DA 00124).

331.3 EFFECTS OF Δ^{9}-TETRAHYDROCANNABINOL (THC) ON THE RESPONSE OF THE INTERMEDIOLATERAL CELL COLUMN TO STIMULATION OF CENTRAL PRESSOR SITES. Michael J. Hosko and Douglas M. Wilkison*. Dept. of Pharmacology and Toxicology, Med. College of Wisconsin, Milwaukee, WI 53226.

THC induced hypotension and bradycardia in experimental animals is mediated through central mechanisms. It has been proposed that THC depresses central sympathetic outflow and/or increases central parasympathetic activity by actions within the medulla and pons region. To investigate the effect of THC on sympathetic outflow, the actions of THC on the blood pressure response to stimulation (100 Hz, 10 sec) of central pressor sites were compared to actions on multiunit activity of intermediolateral cell column (ILC) evoked by paired-pulse stimulation of the same pressor sites in α-chloralose-anesthetized cats. Pressor sites in hypothalamus, mesencephalic reticular formation (MRF), fastigial nucleus and medullary lateral reticular nucleus were studied. Ipsilateral, right side, ILC was explored between T_1 and T_4 for units and/or field potentials evoked by stimulation of several sites as well as by the depressor response to histamine. In these experiments heart rate was reduced 20% and blood pressure lowered 40% by 2 mg/kg THC. The ILC response and the pressor response to stimulation of fastigial nucleus was unaltered by THC. The pressor response to MRF stimulation was not changed by THC, however the ILC response was depressed. Similar effects were observed to stimulation of medullary lateral reticular sties. ILC unit activity evoked by stimulation of either the periventricular hypothalamus or the ventral lateral hypothalamus was depressed by THC. The pressor response to low level stimulation of lateral hypothalamus was also depressed, conversely, the pressor response to maximal stimulation of periventricular hypothalamus was increased. THC had no effect on the pressor response to ILC stimulation.

These data suggest that THC actions on sympathetic outflow are dependent on the site of stimulation as well as the mode of stimulation and is not the result of depression of the preganglionic neurons but is likely the result of complex changes in central regulation. This work is supported by NIDA grant R01 DA 00124.

331.4 d-AMPHETAMINE PRODUCES A LONG-LASTING POTENTIATION OF PURKINJE CELL RESPONSE TO IONTOPHORETICALLY APPLIED GAMMA-AMINOBUTYRIC ACID. A.J. Michael, B.D. Waterhouse, D.J. Woodward, Dept. Cell Biology, Univ. Tx. Health Sci. Ctr., Dallas, Texas 75235.

The present study was conducted to investigate the action of d-amphetamine sulphate (d-A) on rat cerebellar Purkinje (P) cells. Extracellular unit activity of P cells was recorded with multibarrel glass micropipettes. Drug-response histograms collected before, during, and after d-A iontophoresis were used to quantitatively evaluate d-A effects on P cells during periods of spontaneous discharge and during inhibitory responses to microiontophoretic pulses (10 sec. duration, 40 sec. intervals) of gamma-aminobutyric acid (GABA).

In 43 of 44 (98%) neurons d-A suppressed spontaneous discharge at currents ranging from leakage to 40 nA (mean=14 nA). Upon termination of d-A iontophoresis, spontaneous firing rate returned to control levels within one minute in 36 of the 43 (84%) cells, and in less than eight minutes in the remaining seven (16%) cells. In 36 of 45 (80%) units d-A , at currents ranging from leakage to 40 nA (mean=10 nA), augmented GABA-mediated inhibitions of P cell discharge relative to changes in spontaneous activity. In 22 of the 36 cases d-A caused a marked potentiation of the GABA-induced inhibition at microiontophoretic doses which caused little or no change in spontaneous discharge.

The potentiation of GABA inhibition by d-A was frequently long-lasting. In 11 of the 36 cases, GABA responses remained larger than control for five to 15 min. following d-A application. An additional 11 neurons were monitored for periods of 10 to 60 min. with no apparent recovery from d-A effects.

These data support previous findings that iontophoretic doses of d-A can suppress spontaneous discharge of P cells. Furthermore, the data suggest that doses of d-A that are subthreshold for suppression of spontaneous firing rate can produce an augmentation of GABA-mediated inhibition of P cell activity. A similar potentiation of GABA action on cerebellar P cells has been observed during norepinephrine (NE) iontophoresis and following locus coeruleus stimulation. Biochemical investigations indicate that d-A releases endogenous NE from nerve terminals. Nevertheless, the mechanism of the d-A sympathomimetic effect reported here remains to be clarified. An implication of these findings is that d-A may produce its behavioral effect by inducing a long-lasting change in neuronal responsiveness to amino acid neurotransmission.

(Supported by NIDA DA-02338 to DJW and NIH NS18081 to BDW and an award from the Biological Humanics Foundation.)

331.5 DISCRIMINATIVE STIMULUS PROPERTIES OF COCAINE: TOLERANCE AND CROSS-TOLERANCE CHARACTERISTICS. D. M. Wood*, M. W. Emmett-Oglesby and H. Lal. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, Texas 76107.

Rats were trained to discriminate an injection of cocaine, 5 mg/kg, from an injection of saline, using a two-lever choice procedure in which food reinforcement occurred for each 10 responses on the correct lever: one lever was always correct after cocaine injection, and the other lever was always correct after saline injection. After training, cocaine was generalized to the cocaine lever in a dose-dependent fashion, with an ED50 of 3.8 mg/kg. Methamphetamine was also generalized to the cocaine lever, but phenethylamine (PEA) was only partially generalized: 50% of subjects selected the cocaine lever at 80 mg/kg PEA, and higher doses produced non-responding. Cocaine was injected every 8 hours, 20 mg/kg, and the discriminability of 5 mg/kg cocaine was tested every other day. As compared to generalization testing prior to chronic administration, when the cocaine lever was selected by 63% of subjects tested at this dose, on days 2, 4 and 6 of chronic administration the percentage of subjects selecting the cocaine lever was 50%, 40% and 25%, respectively. Redetermination of the generalization curve after day 6 showed a shift to the right with an ED50 of 10.6 mg/kg. In contrast to these data showing tolerance to the discriminative stimulus properties of cocaine, no tolerance developed to the disruptive behavioral effects of cocaine on operant responding as measured by the time required to obtain the first reinforcement in each session. Tolerance to the discriminative stimulus properties of cocaine conferred cross-tolerance to the discriminative stimulus properties of methamphetamine. However, no evidence for cross-tolerance to PEA was obtained. These data indicate that tolerance can develop progressively to the discriminative stimulus properties of cocaine in a short period when it is administered chronically, but this tolerance is not reflected in behavioral measures of operant responding. Cross-tolerance to methamphetamine suggests a common mechanism mediating tolerance to the discriminative stimulus properties of both drugs.

(Supported by AOA Grant 82-11-045)

331.6 KAINIC ACID LESIONS OF THE NUCLEUS ACCUMBENS DISRUPT COCAINE SELF-ADMINISTRATION IN THE RAT. K.A. Zito*, G. Vickers* and D.C.S. Roberts. Department of Psychology, Carleton Univ., Ottawa, CANADA, K1S 5B6.

There is increasing evidence to indicate that cocaine produces its reinforcing effects by acting as an indirect agonist at the dopamine (DA) receptor. 6-Hydroxydopamine (6-OHDA) lesions of the nucleus accumbens have been shown to cause extinction of cocaine self-administration behavior. A similar effect is achieved by destruction of DA cell bodies in the ventral tegmental area (VTA) which innervate the accumbens and other forebrain areas. However, the depletion of DA in the accumbens following VTA lesions does not correlate with the change in cocaine intake, suggesting that DA innervation of some other anterior structure or combination of structures may be critical. We therefore sought to determine the exclusive involvement of cell bodies in the accumbens by employing the neurotoxin kainic acid, which specifically lesions neuronal perikarya while leaving fibers of passage intact.

20 male Wistar rats were implanted with chronic indwelling jugular cannulae and allowed to self-administer cocaine (0.75 mg/kg/inj.) on a continuous reinforcement schedule for 4 hr/day. After self-injection had stabilized, animals received either bilateral kainic acid (0.5 ug/0.5 ul) or vehicle infusions (N=6) into the accumbens. Following a 4 day recovery period, animals were again given the opportunity to self-administer cocaine for a period of approximately 2 weeks or until stable performance was observed.

Following the kainic acid treatment six rats lost considerable weight, some displaying behavioral seizures, and were therefore excluded from the study. Histological examination revealed that these animals had sustained extensive damage to the striatum. Complete lesions to the accumbens were possible without such adverse effects. Although considerable variability was observed in the amount of cell loss to the accumbens, the degree of cellular degeneration in this nucleus was observed to correlate with both rate and pattern of cocaine self-administration. Animals with minimal cell damage to the accumbens displayed only minor disruptions of their prelesion intake, whereas animals with complete lesions displayed a drastic decline in response rate and often ceased to respond altogether. Taken together these results suggest that the nucleus accumbens does indeed play a necessary role in the normal expression of cocaine reward. (Supported by the Medical Research Council Grant MA-7374).

331.7 DOSE-RESPONSE EFFECTS OF METHAMPHETAMINE-PRODUCED LOCATION PREFERENCE. P.M. Duncan, K. Saunders* and P. Byerly*. Psychology Department, Old Dominion University, Norfolk, VA 23508.

Sherman, et al. (Pharmacol. Biochem. Behav., 13:597, 1980), report that rats prefer a distinctive place previously associated with d-amphetamine (1.4 mg/kg) treatment, relative to a place where they were never drugged. In the present experiment 6 doses of methamphetamine (MA) were used to investigate dose effects on place preference. Eighty-four male Long-Evans rats were given IP injections (I) of MA and then confined for 30 minutes in one side of a shuttle box. Next day they were not injected (NI), and were placed in the other side for 30 minutes. Injection and non-injection days were alternated until 5 injections had been given. Two groups of 7 rats each were used for each MA dose. One side of the shuttle box had a black plastic floor and walls with vertical black and white stripes (BW). The other side had unpainted plywood walls (W). At each dose, one group was placed in the W side on I days, and in the BW side on NI days. The other group at each dose had the opposite drug-place pairing. On the 11th day the partition separating the BW and W sides was removed, and saline injection given just prior to a 20 minute side preference test. Time spent in the W side and number of side crossings were recorded. On the 12th day a second preference test was given 20 minutes after injection of MA at the dose given initially.

MA dose	0	.25	.50	1.0	3.0	6.0 mg/kg
Groups drugged in:	Mean total seconds spent in wood side					
wood side	608	750	819	926	806	915
BW side	658	886	573	612	470	419

ANOVA of preference data (nondrugged test day) showed no significant dose effect, but significant ($p<.005$) place and place-dose interaction effects. MA at doses ranging from .5 to 6.0 mg/kg. clearly produced preference for the drug-paired location. The pattern of group means suggests a trend for increasing place preference as a function of MA dose, but preferences were not significantly different among the effective doses. No evidence of an aversion to the drug-paired location was seen. The marked difference in overt behavior produced by MA over this dose range was indicated by significantly more side crossings during the drugged test at the .5 mg (MN=27), and fewer at the 6.0 mg. dose (MN=3.6), compared to the 0 dose (MN=13). The place-preference paradigm may be useful in investigating the conditioned effects of drug treatments at high doses which impair or prevent the emission of operant responses.

331.8 EFFECTS OF SEROTONIN (5-HT) RECEPTOR ACTIVE AGENTS ON d-AMPHETAMINE SELF-ADMINISTRATION IN CONTROL AND MFB LESIONED RATS. A.P. Leccese & W.H. Lyness. Department of Pharmacology, Texas Tech University Health Sciences Center., Lubbock, TX.

While the role of dopamine in psychomotor stimulant self-administration is well documented, relatively little is known of the role of 5-HT containing neurons. Previous work has shown that depletion of brain 5-HT increases the frequency of d-amphetamine self-administration. This report sought to confirm the role of 5-HT using agents reported to affect 5-HT receptor function. Rats in which the forebrain 5-HT projections were destroyed (5,7-DHT MFB lesions) were employed to ascertain effects not mediated by 5-HT neurons. Both control and lesioned groups were allowed access (8 hr/day) to an apparatus which delivered .125 mg/kg/inj d-amphetamine through indwelling venous catheters. While both groups acquired stable self-injection rates with 7 days, the 5,7-DHT lesioned group consistently self-administered more drug/test session. L-tryptophan pretreatment (100 mg/kg i.p.) reduced self-injection markedly in controls for the initial 4 hrs of the test session but did not affect the MFB lesioned group. Fluoxetine (5,10 mg/kg), a 5-HT reuptake inhibitor, similarly reduced response patterns in controls and had no effect in the MFB lesioned group. Quipazine (5,10 mg/kg) also reduced d-amphetamine self-injection in a dose-related manner in controls.

Pretreatment with 5-HT receptor antagonist cyproheptadine (5,10,20 mg/kg) unexpectedly and profoundly reduced drug self-injection rates in control rats. MFB lesioned rats, however, displayed rapid lever pressing behavior resembling an extinction response. The frequency of such was that 3 of 6 animals in this group died of d-amphetamine overdose. Methysergide pretreatment yielded comparable findings. While this agent only transiently reduced amphetamine self-injection at 10 mg/kg in control rats, like cyproheptadine pretreatment, the response of MFB lesioned rats was accelerated (continuous bursts of lever pressing).

The actions of the putative 5-HT agonists in control animals support our original hypothesis that 5-HT neurons are of import in the perception of reward with a positive reinforcing drug like d-amphetamine. It would appear 5-HT's action is independent of dopamine. Rats in which 5-HT containing nerve terminals were destroyed might be predicted to show no response to agents like tryptophan and fluoxetine, which depend on 5-HT presynaptic integrity for their actions. The activity of cyproheptadine and methysergide are unexplained at present. Neurochemical & behavioral analyses will be presented which suggest pharmacologic actions other than 5-HT receptor blockade may be responsible.

This work was supported by NIDA grant ROI-02997.

331.9 TOLERANCE TO LSD-INDUCED BEHAVIOR IN PRIMATES IS NOT DEPENDENT ON ENVIRONMENT R.F.Schlemmer, Jr., C. Nawara*, W.J. Heinze*, J.M. Davis, C.D. Advocat. Ill. State Psychiatric Inst. & Department of Pharmacology, University of Illinois, Chicago, Illinois 60612.

The influence of environmental contingencies on tolerance has been particularly well characterized with respect to the opiates, alcohol, & sedative-hypnotics. One class of drugs which has not been examined in this regard is the hallucinogens. A rapid partial tolerance develops to the hallucinogenic effect of most hallucinogens notably d-lysergic acid diethylamide (LSD). As yet, the mechanism responsible for this tolerance is unknown. Interestingly, despite the development of tolerance to hallucinogen related behavior in animals, tolerance fails to develop to LSD-induced suppression of raphe cell firing, an action which has been suggested as a mechanism by which hallucinogens elicit their psychotomimetic effect. This dichotomy raises a question concerning the possible involvement of non-pharmacologic mechanisms in the development of tolerance to hallucinogens. The purpose of this study was to examine this possibility by determining whether or not tolerance to the behavioral effect of LSD was specific to the environment in which the drug was given. The paradigm used for the study was observation of open field behavior in members of a primate social colony. Acute administration of LSD and other hallucinogens to selected members of primate social colonies induces several significant behavioral changes. One emergent behavior, limb jerks - myoclonic spasms of the extremities, is specifically elicited by hallucinogenic drugs. As is the case with the hallucinogenic effect in humans, tolerance develops to LSD-induced limb jerks in primates by the second day of daily repeated administration. In the study, LSD, 0.01 mg (base)/kg, was administered i.m. once daily for 5 consecutive days to each member of a stable social colony of 4 adult Stumptail macaques. During the first 2 days, the colony was housed in their large home group cage (1.5 x 2.5 x 3.5m). On days 3-5, all monkeys were moved to smaller individual cages in a different room. Following a 2-week drug wash-out period, the experiment was repeated with the animals remaining in their home group cage throughout the 5-day treatment period. Behavioral observations were conducted by 2 experienced "blind" observers for 60 min. each day beginning 15 min after LSD injection. Tolerance developed to the limb jerk response between the first & second drug injection. This decrement was maintained after the third injection when the administration context was changed. During the 2-week drug-free interval, the number of limb jerks returned to nontolerant levels. A comparable decrement in limb jerks occurred during the second 5-day treatment period. These results show that tolerance of the limb jerk response elicited by LSD is not situation specific but depends on the continuous presence of drug, regardless of prevailing environmental conditions.

331.10 TOLERANCE DEVELOPS TO 5-MeODMT-INDUCED BEHAVIORAL CHANGES IN MONKEYS UPON FREQUENT, REPEATED ADMINISTRATION. W.J. Heinze*, R.F. Schlemmer, Jr., J.A. Retallack*, C.A. Sink*, C. Nawara*, J.M. Davis. Illinois State Psychiatric Institute, Chicago, IL 60612.

A rapid partial tolerance develops to the hallucinogenic effects and physiological changes induced by most hallucinogens, in humans and animals upon repeated administration. Tolerance has not been reported to develop to any 5-MeODMT-induced behavioral or physiological change upon once daily repeated administration to several animal species. We reported that 5-MeODMT induces several significant behavioral changes in selected members of primate social colonies. Tolerance fails to develop to any 5-MeODMT-induced behavioral change upon once administration for 12 days. One behavior, limb jerks (myoclonic spasms of the extremities) appears to be specifically induced by hallucinogens. Tolerance is seen with limb jerks induced by hallucinogens which also show tolerance in humans, such as dLSD, Mescaline, and DOM. Unlike these hallucinogens, 5-MeODMT has a fast onset of action of less than 5 min, peaks within 30 min, and has a duration of action of approximately 1 hr in monkeys. The purpose of the present experiment was to determine whether tolerance would develop to this fast-acting hallucinogen, if it was administered more frequently than once a day. The subjects for the study were 4 female Stumptail macaque monkeys who comprised a stable social colony. The study began with observation of undrugged behavior (baseline), followed by drug treatment in a crossover design. Each monkey in the colony received an i.m. injection of saline or 5-MeODMT, 0.25 mg/kg, every 25-35 min for 9 hr throughout the day. Five 60 min observation sessions were conducted every other hr by "blind", experienced, primate observers who recorded colony behavior using standard techniques, beginning 5 min after injection. Partial tolerance developed to 5-MeODMT-induced body shakes within 3 hr, and also developed to 5-MeODMT-induced limb jerks and increased checking within the 9 hr treatment period. However, tolerance failed to develop to 5-MeODMT-induced reduction in social grooming and self grooming within the 9 hr period. The pattern is similar to the pattern of tolerance developed to other hallucinogens such as LSD tested in this species. Also, there was a significant increase in resting behavior in the latter stages of treatment which was not seen upon acute administration. Interestingly, tolerance was still evident when 5-MeODMT was given the next day, 17 hr after the last injection. The result of this study demonstrate, that the frequency of drug administration is critical to the development of tolerance to hallucinogens. These data also raise serious doubts as to the possible role of 5-MeODMT as an endogenous psychotogen since tolerance develops to 5-MeODMT upon continuous exposure over a short period of time.

331.11 PHENCYCLIDINE, COCAINE AND AMFONELIC ACID ACTIONS AT TETRABENAZINE SENSITIVE INTRANEURONAL SITES. S.P. Bagchi. Rockland Research Institute, Orangeburg, New York 10962.

Recent results (Bagchi, Biochemical Pharmacol. in press) indicate that phencyclidine (PCP), cocaine and amfonelic acid (AFA) stimulants interact with tetrabenazine (TBZ). Also, these drug interactions, which inhibit dopamine (DA) formation from phenylalanine, appear to be at the level of intraneuronal storage vesicles. Since nerve-ending homogeneity of phenylalanine and tyrosine hydroxylating activities has never been established, the present work has investigated if any change of DA formation may result from the stimulant - TBZ interactions with tyrosine as the precursor. For these experiments, nerve-ending rich fractions (P_2) from rat brain caudate nuclei were incubated for 10 min (37°) with labelled tyrosine in tris buffer (pH 7.4) containing various salts, pargyline, glucose and sucrose. Post-incubation separation of the particulates from the medium was by Millipore filter followed by the analysis of the seperated fractions for labelled DA. Particulate formation and release of labelled DA were determined in the presence of TBZ alone and with the stimulants. Results indicate that TBZ (0.022 to 5.4 uM), in a concentration-related manner, inhibited the formation of DA and enhanced its release. In the absence of TBZ or at its lower concentrations (0.022 to 0.09 uM), addition of either PCP, cocaine, or AFA (0.91 to 36.4 uM) had no effect on the formation of DA but enhanced its release. TBZ at higher concentrations (0.36 to 5.4 uM), however, revealed additive inhibitory effects of the same drugs upon DA formation. Only synthesis stimulation was seen with amphetamine (9.1 uM) at any TBZ level. Present results and other phenylalanine data indicate that, at higher TBZ levels (1.8 to 5.4 uM), inhibitions of DA formation by PCP, cocaine and AFA may result with either precursor. At the lower TBZ levels (0.022 to 0.36 uM), however, these stimulants may show no effect or inhibitions with tyrosine but stimulations with phenylalanine. Reserpine (0.022 to 1.8 uM), unlike TBZ, revealed only inhibitory effects of these stimulants with either precursor. In conclusion, results with tyrosine as precursor confirm PCP, cocaine and AFA actions upon TBZ sensitive DA storage vesicles. Furthermore, there may be heterogeneous populations of the vesicles with differential sensitivities to the stimulants. This work was kindly supported by the Office of Mental Health, State of New York.

331.12 ACCUMULATION AND BINDING OF PHENCYCLIDINE IN MELANIN BEARING TISSUES. Irwin Fand, William P. McNally and Emmeline Edwards. Lab. of Cellular Neurobiology, L.I. Res. Inst., SUNY at Stony Brook, Stony Brook, NY 11794.

The identification of tissue depots for phencyclidine(PCP) with possible slow release of this psychotomimetic is important in the search for etiologic factors accounting for the persistence of long-lasting PCP effects in humans.

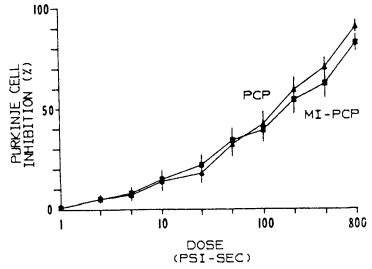
A previous study by Misra et al. (Res. Comm. Chem. Path. Pharm., 24 : 431, 1979) established the in vitro binding affinity of ^{3}H-PCP to synthetic melanin. The present study was undertaken to describe the binding charateristics of PCP within melanized tissues in vivo, thereby providing a depot for possible slow release of drug. Using a model in vivo system, highly melanized melanoma borne in BALB/c mice demonstrated strong and selective accumulation of ^{3}H-PCP in the tumor. The accumulation of radioactivity equivalents of ^{3}H-PCP, examined in whole-body autoradiograms, was observed as early as 20 mins. after a single tail-vein injection and ^{3}H-uptake in the melanoma persisted in observable but significantly lower levels within melanoma tissue at 18 hrs. after a single drug dose.

In vitro binding studies, performed with cultured cells, generally confirmed the previously described in vivo results. ^{3}H-PCP assays were performed with cultured human melanoma cells (MEWO line) and human diploid skin fibroblasts. Non-specific binding was determined with N-Ethyl-1-phencyclohexylamine (PCE) as displacer and was found to be within 5-20% of the total binding. The binding was highly saturable and Scatchard parameters indicated a single population of sites. Binding affinity constants (expressed in nM), on the order of 50-60 nM, did not differ between the cell lines examined. However the maximal number of binding sites (B_{max}) for the melanin-rich MEWO cells (11 Pmole/mg protein) exceeded the level of binding within the amelanotic human skin fibroblasts by 63%. Fluorometric determinations of melanin in MEWO cells yielded values of 4×10^{-4} mg/mg wet weight of cells.

These combined in vivo and in vitro data argue for depot localization of PCP in melanin-bearing cells. These findings also suggest that similar melanin sites such as locus coeruleus and substantia nigra may sequester PCP to allow slow release of drug, helping to explain the long-lasting psychosis associated with PCP.

331.13 ELECTROPHYSIOLOGICAL DETERMINATIONS OF STRUCTURE-ACTIVITY RELATIONSHIPS OF PHENCYCLIDINE DERIVATIVES. K. Pang*, S. Johnson*, R. Freeman*, B. J. Hoffer* and S. Maayani† (SPON: S. G. Beck). Dept. of Pharmacology, Univ. of Colorado Health Science Center, Denver, CO 80262, and †Dept. of Pharmacology, Mt. Sinai School of Medicine, New York, NY 10029.

The effects of phencyclidine (PCP) and three of its derivatives (m-amino-PCP, m-nitro-PCP, and PCP methiodide) were studied on rat cerebellar Purkinje (P) cells. Previous studies using behavioral criteria have shown that these derivatives have the following relative potencies:amino-PCP > PCP >> nitro-PCP. PCP methiodide (MI-PCP) was reported to be inactive. In order to determine if differential access into the CNS could account for the order of potencies seen in the behavioral studies, these drugs were administered locally onto P cells by pressure microejection from multibarrel pipettes and also administered by i.p. injection. PCP and its derivatives generally caused a decrease in P cells spontaneous activity. The order of potencies after parenteral administration of drugs was the same as found in the earlier behavioral studies: amino-PCP > PCP >> nitro-PCP. PCP methiodide was also found to be inactive electrophysiologically after systemic administration. In contrast, a different order of potencies was found when the drugs were locally applied onto the P cells: amino-PCP > PCP = nitro-PCP = PCP methiodide. These results suggest that the differences in behavioral and electrophysiological potencies of PCP derivatives seen after systemic administration may be due to differences in the entrance of the drugs into the CNS. In addition, since PCP has previously been shown to interact with noradrenergic synapses in the cerebellum, the strong correlation between behavioral and electrophysiological results after parenteral administration of PCP derivatives suggests a noradrenergic involvement in the behavioral effects of PCP.



331.14 EFFECTS OF COCAINE ON THE CEREBRAL ACCUMULATION OF KETAMINE AND ITS METABOLITE I IN THE MOUSE. Mary E. McKenna* and Christina VanderWende. Rutgers-The State University, Box 789, Piscataway, N.J. 08854.

The loss of righting reflex in the mouse after administration of ketamine, a dissociative anesthetic, has been shown to be potentiated instead of antagonized by cocaine, a stimulant. Since ketamine is very lipid soluble and is known to rapidly accumulate in cerebral tissue, it was felt that quantitation of ketamine and its main metabolite (metabolite I) in mouse brain after administration of ketamine or ketamine plus cocaine might help elucidate this seeming contradiction.

Male albino, Swiss Webster mice were administered intraperitoneally 100 mg/kg ketamine HCl or 100 mg/kg ketamine HCl followed immediately by 30 mg/kg cocaine HCl. Of the 30 mice per group, 5 mice were sacrificed at 1, 5, 10, 15, 30 and 60 minutes after dose administration by decapitation. Brains were removed, rinsed and homogenized in 0.1N HCl to yield a 1% homogenate. Aliquots of each sample were extracted and analyzed for ketamine and its metabolite I by a modification of the Chang and Glazko procedure employing a G.C. equipped with an electron capture detector (Chang, T. and Glazko, A.J., Anesthesiology, 36: 401-404, 1972).

Duration of the loss of righting reflex and ketamine brain AUC was increased two-fold and 1.4 fold respectively in the animals administered ketamine plus cocaine as compared to the ketamine alone controls. There was also a slight shift to the right in the T_{max}, in the former vs the latter. However, the peak brain concentrations were essentially the same for both groups. Although there was no significant difference in the AUC or peak brain concentrations of metabolite I for either group, there was a significant difference in the concentrations at the later time points.

Onset time and sleep time were both observed at ketamine concentrations of approximately 24 ug/g brain tissue. For ketamine controls ketamine concentrations below this level occurred at approximately 12 min. and at approximately 27 min. for the cocaine treated animals. Thus the brain concentration data paralleled the observed pharmacological effect. Based on the data presented, a metabolic explanation for the potentiation of ketamine by cocaine is offered.

331.15 EFFECT OF IN VIVO ADMINISTRATION OF LSD ON INITIATION OF PROTEIN SYNTHESIS IN A CELL-FREE SYSTEM DERIVED FROM BRAIN. J.W. Cosgrove and I.R. Brown. Department of Zool. University of Toronto, West Hill, Ontario MIC 1A4 Canada.

We have previously shown that the intravenous injection of LSD to young adult rabbits induces a disaggregation of brain polysomes to monosomes (Holbrook, L.A. and Brown I.R., J Neurochem., 27: 77, 1976), and a decreased incorporation of [35S]methionine into brain proteins both in vivo (Freedman, M.S. et al., Brain Res., 207: 129, 1981) and in an initiating cell-free protein synthesis system derived from brain postmitochondrial supernatant (PMS) (Cosgrove, J.W. et al., J. Neurochem., 36: 1037, 1981). These phenomena are transient wtih a maximal effect 1 hr after LSD administration and a return to control levels 6 hr after drug injection. Our previous results using in vivo and in vitro experimental approaches suggested that there was a lesion at the initiation stage of brain protein synthesis. We now analyze the effect of in vivo administration of LSD on subsequent initiation of protein synthesis in the cell-free protein synthesis assay derived from brain. We have previously reported that this system has the capacity to initiate protein synthesis in vitro (Cosgrove, J.W. and Brown, I.R., J. Neurochem., 36: 1026, 1981). Three inhibitors of initiation, edeine, poly rI, and aurintricarboxylic acid (ATA) were used to demonstrate a reduction in initiation-dependent amino acid incorporation in the brain cell-free system prepared 1 hr after LSD administration. At this time there was a measurable decrease in the formation of 40S and 80S initiation complexes in vitro. This decrease was observed using either [^{35}S]methionine or [^{35}S]methionyl-tRNA to label initiation complexes. Analysis of the methionine amino acid pool after LSD administration indicated that there was no change in the methionine levels. Analysis of the formation of initiation complexes in the brain cell-free protein synthesis assay prepared 6 hr after LSD administration indicated that there was a return to control levels at this time. These results indicate that the effects of LSD on specific steps in the initiation process are reversible and coincide with the time course of recovery to control protein synthesis levels which we have previously measured by other in vivo and in vitro approaches. (Supported by grants from the Medical Research Council, Canada).

332.1 ORGANIZATION OF DORSAL RAPHE PROJECTION NEURONS AND SEROTONERGIC INNERVATION OF THE RAT TECTUM. J.C. Baack*, R.D. Dey*, and B.D. Waterhouse. (SPON: R.A. Galosy). Dept. of Cell Biology, Univ. Tx. Health Sci. Ctr., Dallas, TX 75235.

The purpose of the present study was to examine the distribution of the 5-hydroxytryptamine (5-HT)-containing nerve fibers in the rat tectum and to determine the intranuclear distribution of dorsal raphe (DR) neurons which project to the rat superior colliculus. The 5-HT containing fibers in the superior and inferior colliculi were visualized using immunohistochemical techniques. Retrograde transport of HRP from tectal injection sites was employed to examine the distribution of DR (B6+B7) neurons projecting to the superior colliculus. For immunohistochemistry brains were sectioned sagittally and coronally through the tectum. Cryostat sections, 10um thick, were incubated with 5-HT antiserum (diluted 1:100-1:300) for 30 min at 37° in a humid chamber. Control sections were incubated with 5-HT antiserum absorbed with 100ug of 5-HT. The sections were rinsed in PBS-TX, then covered with FITC-labeled goat antirabbit IgG (1:30) and examined with a fluorescence microscope. For retrograde transport studies, solutions of HRP (20%) were pressure injected unilaterally into the superior colliculus after removing the overlying cortical tissue by suction. After survival times of 24 hrs the brainstem was sectioned (40um) in the coronal plane and the tissue reacted according to the TMB method. Sections were examined by light microscopy after counterstaining with neutral red. Nerve fibers with 5-HT like immunoreactivity were observed in both the superior and inferior colliculi. When viewed in the sagittal plane, the 5-HT fibers were oriented tangentially to the surface of the tectum. The heaviest concentrations of 5-HT fibers were observed in the dorsal 200 um along the midline of the tectum. In the DR nucleus, the majority of HRP-filled cells were found ipsilateral to the injection site, adjacent to the midline. These labeled neurons were concentrated in the rostral 2/3 of the nucleus. In the coronal plane, labeling was confined to the ventral portion of the DR, between the medial longitudinal fasciculi. Previous studies indicate that cells in this zone of the DR also project to the occipital cortex. In summary, this data suggests that 5-HT fibers in the rat tectum exhibit an organized distribution. Moreover, DR cells which project to the superior colliculus and occipital cortex are localized in the same region of the DR nucleus. This overlapping organization of projection cells and the non-random distribution of 5-HT fibers in the tectum may be functionally significant since both the superior colliculus and occipital cortex process visual information. (Supported by NINCDS NS-18081 and the Klingenstein Foundation to BDW).

332.2 MIDLINE BRAINSTEM PROJECTIONS TO THE MAIN OLFACTORY BULB OF THE RAT. S. Schumacher*and M.T. Shipley (Spon: N. L. Wiech). Dept. of Anat. and Cell Biol., Col. of Med., Univ. of Cincinnati, Cincinnati, OH 45267

Centrifugal afferents from the brainstem to the main olfactory bulb (MOB) have been noted in several species but these projections have generally been held to be relatively minor. We have found (Shipley et al., this volume) that one of these, the noradrenergic projection from locus coeruleus (LC) is quite large; at least 40% of all LC neurons terminate in MOB. In view of this surprisingly heavy projection from the pons, we have re-examined other brainstem projections to MOB.

Injections of wheat germ agglutinin conjugated to horseradish peroxidase (1%, 25-100 nl) were made in MOB of adult Sprague Dawley rats. The animals survived 24 hrs and the brains were processed by a TMB method.

The dorsal raphe nucleus (nDR) was the most heavily labelled midline structure in the brainstem. Preliminary counts indicate that 150-200 neurons were labeled. A few positive cells appeared to lie outside the lateral cytoarchetectonic limits of nDR. Far fewer cells were labeled in nucleus centralis superior (nCS; median raphe). A previously undescribed projection from nucleus raphe pontis was also labeled. This projection was heavier than that from nCS. In some cases, lightly labeled neurons were found in parts of the mesopontine tegmentum, which were not readily assignable to the raphe complex.

The present findings indicate that midline brainstem projections to MOB are more extensive than previously recognized. Most of these midline cells lie in nuclei of the raphe complex, nuclei which contain most of the brain's serotonergic neurons. Serotonergic terminals have been reported in MOB (Dahlstrom, et al., 1965). The present results suggest that at least three raphe nuclei may participate in a brainstem→MOB serotonergic pathway. Since it is not known whether all neurons of all raphe nuclei are serotonergic, however, it will be necessary to combine retrograde and histo-immunocytochemical methods to arrive at a better understanding of these surprisingly rich midline projections to MOB.

Diverse functions have been proposed for raphe serotonergic neurons but their precise physiological significance has remained illusive. MOB is only one synapse removed from primary olfactory receptors. This circumstance, plus the orderly laminar arrangement of MOB neurons, dendrites and terminal systems, make it an attractive structure for investigating cellular-molecular aspects of raphe function.

Supported by NIH NS-1849, NS-19730 and USAMROC DAMD 17-82-C-2272.

332.3 NUCLEUS RAPHE MAGNUS AND ITS SEROTONERGIC NEURONS: QUANTITATIVE ANATOMICAL STUDIES IN THE RAT, M.W. Wessendorf and R.P. Elde, Dept. Anatomy, Univ. Minnesota, Minneapolis, MN 55455

The influence of nucleus raphe magnus (NRM) on spinal autonomic and motor function, and on inhibition of afferent transmission has been intensively studied. However, there has been no comprehensive anatomical study of NRM in rat. This study uses Nissl and immunocytochemical staining to describe NRM and its serotonergic component as they exist in the rat.

In Nissl studies, rats 80-200 g. were killed by perfusion with Zamboni fixative, and 50 µm frozen sections were cut and stained with cresyl violet. NRM was found to extend from the rostral border of the inferior olive to the level of the pontine gray, a distance of about 2.6 mm. In its mid portion, its dorsoventral extent averaged 1268±108µm (S.E.M.), and its lateral extent from the midline averaged 139±8 µm. Within NRM, 4 types of neurons were classified among 428 neurons examined: triangular (mean major axis±S.E.M.= 29.5±0.7µ; mean minor axis±S.E.M.= 15.4± 0.3µ), fusiform (29.3±0.3µ; 10.2±0.3µ), multipolar (27.4±0.9µ; 16.2± 0.5µ), and oval (20.5±1.0µ; 12.3±0.5µ).

In immunocytochemical studies, rats were pre-treated with 100 mg/kg tryptophan and 20 mg/kg tranylcypromine to increase intraneuronal 5HT levels. The brainstem was cryostat sectioned and processed for 5HT-like immunoreactivity (5HT-LI) using indirect immunofluorescence. Sections were counter-stained with ethidium bromide, a fluorescent Nissl stain. When the Nissl profiles of 5HT-LI neurons were examined, it was found that the same 4 types of neurons were observed as in the general population. No significant differences were observed between the dimensions of the 5HT-LI neurons and NRM neurons as a whole, except for that of the minor axis of the fusiform neurons (12.5±0.4µ, p<.001). Thus it appears that serotonergic neurons are only slightly different from the general population in the sizes and shapes of their somata.

The ratio of 5HT-LI neurons to total neurons in NRM was counted in 4 rats. The value ranged from 4.0% to 7.8%, averaging 5.8%. By combining the proportion of 5HT-LI neurons given here with previously reported values for the proportion of serotonergic neurons which descend to the spinal cord, and for the proportion of spinally-projecting neurons in NRM which contain 5HT (Bowker et al, 1981), the proportion of NRM neurons which descend to the spinal cord can be inferred. The value is under 8%.

It is concluded that the majority of neurons in NRM are not serotonergic. The hypothesis that NRM has a relatively small spinal projection is currently being tested.

(Supported by F32 DA 05226 and DA 02148).

332.4 *DEMONSTRATION OF DOPAMINE AND p-TYRAMINE WITH SPECIFIC ANTISERA AGAINST THESE CATECHOLAMINES.* *M. GEFFARD, Ph. SEGUELA, R.M. BUIJS* and M. LE MOAL, Lab. Neurobiol. Comport., Univ. Bordeaux II, 33076 Bordeaux (France) and *Brain Research Institute, Amsterdam (the Netherlands).(SPON. Prof. M. LE MOAL)*

Dopamine (DA) and p-tyramine (TA) have never been demonstrated directly nor specifically, using immunocytochemical techniques. These latter are known to be particularly useful for the localization of fine processes. Visualization of these molecules was unsuccessful as it was difficult to raise antibodies against small haptens.

We have described (Geffard et al., submitted for publication) a method by which DA and TA have been coupled to the protein carrier by glutaraldehyde in such a way that antigenic determinants of DA or TA were fully presented and the specific antibodies obtained recognized DA or TA with high affinity and good specificity.

Glutaraldehyde is known to couple quickly, irreversibly and predominantly the ε-amino-group of lysine and the amino-group of DA or TA. The result of coupling is signaled by the appearance of double-bonds which may be reduced. The objectives of this study were to keep DA or TA as much as possible in their original molecular form in order to prevent a loss of antigenicity during the testing procedures in the tissues. A radioimmunological method (RIA) was used to demonstrate the time course of immunization, the specificity and affinity of these antibodies. DA, TA and the other catecholamines were conjugated by glutaraldehyde (G) to the ε-amino-group of N-α-acetyl-L-lysine N-methylamide (ALM). It turned out that the anti-DA antibodies or anti-TA antibodies only recognized DA coupled or TA-coupled, but had no affinity for uncoupled DA or TA. Displacement curves established with (^{3}H)DA-G-ALM or (^{3}H)TA-G-ALM showed the same cross-reactivity between DA-G-ALM and NA-G-ALM for anti-DA antibodies, and between TA-G-ALM and DA-G-ALM for anti-TA antibodies. Immunocytochemical results were in good agreement with RIA tests and published data.

In conclusion, sensitive and specific antibodies to amines can be raised provided that : (i) the necessary precaution to preserve the molecular form of the substances are taken; (ii) if a fixative is used to couple the antigen to a protein carrier the same fixative should be used to retain the antigen in the tissue; (iii) for the specificity and affinity tests the antigen should be fixed to a lysine carrier using the same fixation procedure.

332.5 POSTNATAL DEVELOPMENT OF LAMINAR INNERVATION PATTERNS BY NORADRENERGIC AND SEROTONGERGIC FIBERS IN MONKEY PRIMARY VISUAL CORTEX. S. L. Foote, J. H. Morrison, and F. E. Bloom. Lab. Behavioral Neurobiology, Salk Institute, San Diego, CA 92138.

Our previous studies in adult squirrel monkey (Saimiri sciureus) have shown that noradrenergic (NA) and serotonergic (5-HT) fibers in primary visual cortex exhibit complementary laminar innervation patterns. 5-HT fibers are generally restricted to the upper four cortical laminae and are especially dense in layer IV, while the NA innervation is especially dense in layers V and VI, moderate in layers I, II, and III, and virtually absent in layer IV (Morrison et al., PNAS, 79:2401, 1982 and Brain Res. Bull., 9:309, 1982). Subsequent studies have shown distinct, but limited, modifications of these innervation patterns in adult cynomolgus monkey (Macaca fascicularis: Kosofsky and Molliver, Anat Rec, 205:A101, 1983; Morrison et al., in press) and adult Macaca fuscata (Takeuchi and Sano, Anat Embryo, 166:155, 1983).

Since these monoamines, especially NA, have been hypothesized to play an essential role in the developmental "plasticity" of visual cortex organization, we have examined the postnatal development of these innervation patterns. Immunohistochemical methods (Morrison et al., PNAS, 79:2401, 1982) were used to visualize NA and 5-HT fibers in primary visual cortex (area 17) of cynomolgus monkeys of various postnatal ages (1 day; 18 days; 60 days; adult). At birth, a dense 5-HT innervation of layer IV is present along with sparse innervation of other laminae. The full adult pattern of 5-HT innervation becomes evident by 60 days of age. NA fibers are much less dense than 5-HT fibers at all ages although their density gradually increases with age. At birth, very sparse, coarse NA fibers were evident distributed evenly through all cortical layers. By 60 days, the lack of NA fibers in layer IVc which characterizes the adult has become evident due to enhanced arborization of NA fibers in other layers.

With regard to the greater density of 5-HT than NA fibers and the increasing density of both types of fibers with development, our results are compatible with previous biochemical measurements (Goldman-Rakic and Brown, Dev. Brain Res., 4:339, 1982). Our observations indicate that, relative to NA fibers, 5-HT fibers are present in greater density at an earlier age, and are more crucially positioned to play an important role in developmental changes in geniculo-cortical organization. However, these developmental patterns may not reflect any participation in reorganization, but rather the gradual development of some other functional capacity such as the modulation of visual information processing. Supported by USPHS Grants NS 16209 and NS 18023.

332.6 THREE-DIMENSIONAL RECONSTRUCTION OF DOPAMINE NEURONS IN THE MOUSE: STRAIN DIFFERENCES IN REGIONAL CELL DENSITIES AND PHARMACOLOGY. D.C. German, K.L. McDermott, M.K. Sanghera, D.S. Schlusselberg, W.K. Smith, D.J. Woodward, S.G. Speciale and C.B. Saper. Depts. of Physiol., Psychiat., and Cell Biol., U. Texas Health Sci. Cntr., Dallas, TX 75235 and Dept. of Neurol., Washington U. Sch. of Med., St. Louis, MO 63110.

Genetically inbred strains of mice have been found to possess differing numbers of CNS dopamine (DA)-containing neurons. Such differences in midbrain DA cell number have been correlated with behavioral differences. For example, Fink & Reis (Brain Res. 222:335-349, 1981) found that the BALB/cJ mouse has more midbrain DA neurons (@ 20% overall) than the CBA/J mouse, and the former exhibits more spontaneous locomotion and amphetamine-induced stereotyped motor output than the latter. The purpose of the present experiment was to examine where in the three-dimensional space of the midbrain DA neurons the difference in DA cell number occurs, and to examine DA and DOPAC levels in limbic and striatal sites. BALB/c and CBA male mice were decapitated and the brains rapidly removed. Micro-punches were taken from the right and left striatum and nucleus accumbens. HPLC assay was done on DA and DOPAC. Four midbrains from each strain of mice were formalin fixed, sectioned at 30 µm and stained with a tyrosine hydroxylase antibody. Cell locations were microscopically entered (every 4th section) into a digital computer storage system. Although BALB/c mice had approximately 20% more total DA neurons than the CBA, 3-dimensional reconstructions of the distribution of midbrain DA neurons reveal topographic differences in DA cell numbers between the two mouse strains. Furthermore, pharmacological results (ng/mg protein) indicate that CBA mice have significantly more striatal DA and greater apparent DA turnover (DOPAC/DA ratio) than BALB/c mice, and the CBA has more DA (17%) on the left side. In the n. accumbens, however, the BALB has significantly more DA than the CBA but less turnover than in the CBA. These data indicate that regional differences in DA cell numbers/pharmacology need to be considered for an understanding of differences in DA-related behaviors between mouse strains.

		Striatum		N. Accumbens	
		DA	DOPAC/DA	DA	DOPAC/DA
BALB/c	Right	204 ± 10	.052 ± .001	183 ± 17	.099 ± .013
	Left	209 ± 14	.052 ± .002	173 ± 14	.095 ± .012
CBA	Right	268 ± 13	.067 ± .004	164 ± 13	.130 ± .003
	Left	325 ± 16	.067 ± .006	160 ± 10	.124 ± .005

Research supported by grants MH-30546, EY-03327, AA-5198, AA-3901, and Biological Humanics Foundation.

332.7 THREE-DIMENSIONAL RECONSTRUCTION OF LOCUS COERULEUS NEURONS IN THE HUMAN BRAIN. B. Walker*, K.L. McDermott, W.K. Smith, D.S. Schlusselberg, D.J. Woodward, and D.C. German (SPON: C.M. Michael). Depts. of Physiol., Psychiat., and Cell Biol., U. Texas Health Sci. Cntr., Dallas, TX 75235.

The noradrenergic neurons of the locus coeruleus (LC) have been the focus of much neuroscience research. The LC is located in the dorsolateral pontine tegmentum, medial to the mesencephalic nucleus and tract of the trigeminal nerve. These cells have extensive ascending and descending axonal projection systems. For example, these neurons innervate the entire cerebral cortex and cerebellar cortex and send descending axons into the spinal cord. An examination of cortical LC synaptic transmission indicates that norepinephrine acts as a neuromodulator, to amplify certain neurotransmitter systems (for example, GABA). In the human brain, the LC neurons contain neuromelanin pigment. This pigment is a by-product of norepinephrine synthesis and it accumulates in the cells with aging. LC cells also decrease in number with aging. It has been speculated that excessive neuromelanin pigment accumulation results in cell death.

The purpose of the present experiment was to document the 3-dimensional topography of the LC in man and to document regional changes in cell number which accompany aging. Formalin fixed brains were cut, perpendicular to the long axis of the brainstem, at 50 µm thickness throughout the rostral-caudal extent of the nucleus (about 13 mm). Sections were stained with the Schmorl's ferricyanide method, a neuromelanin stain. Every 16th section was entered into a digital computer storage system with image frame buffer. In the first brain examined, a 24 year old right-handed male, there were 1841 cells on the right side and 1911 cells on the left side. Seventeen sections were counted and cell counts increased symmetrically from rostral to caudal. For example, rostrally these were 20-50 cells/side/section and caudally there were 120-200 cells/side/section. This technique for creating precise plots of cell distribution is being employed to reveal the 3-dimensional topography of cell number and regional neuromelanin pigment density across different age groups.

Research supported by grants MH-30546, NS-20030, DA-2338, AA-3901 and the Biological Humanics Foundation.

332.8 SPINAL PROJECTIONS OF THE A-11 DOPAMINERGIC CELL GROUP IN THE RAT. R. L. Stornetta* and P. G. Guyenet (SPON: C. Creutz). University of Virginia School of Medicine, Dept. of Pharmacology, Charlottesville, VA 22908.

The present series of experiments was undertaken to determine the exact contribution of the dopaminergic A-11 group to the innervation of the rat thoracic spinal cord and the distribution of its terminal field at this level. Tissue catecholamine (CA) content of dopamine (DA), norepinephrine (NE) and epinephrine (EPI) was measured with HPLC coupled with electrochemical detection and the distribution of CA terminals in the thoracic cord was determined using the glyoxylic acid technique (de la Torre & Surgeon, Histochem.,49: 81,1976). All experiments were done in animals subjected to intracerebroventricular (ICV) injections of 6-hydroxydopamine (6OHDA) resulting in the disappearance of 95% of spinal NE ($\bar{x}$ ± S.E.M.: control=1405 ± 55 pmoles/g, N= 13; 6OHDA= 70 ± 7, N=9) but sparing entirely the DA innervation. In these animals, DA was thus the dominant CA in the thoracic spinal cord.

Examination of the thoracic cord from NE depleted animals revealed a total loss of CA fluorescence in the ventral horn and a great reduction in the intermediolateral cell column (ILC) and the central canal as compared with control tissue. The CA fluorescence in dorsal horn (DH) was moderately reduced.

The extent of the A-11 DA projection and its terminal field distribution was determined by comparing a series of NE depleted rats (N=6) to an experimental group of NE depleted rats (N=6) subjected to an additional large bilateral electrolytic lesion of periventricular hypothalamus including the A-11 area. The lesion resulted in an average 50% depletion of spinal DA (117 ±10 pmoles/g vs 220 ± 10 for controls) while the NE level was identical to ICV 6OHDA NE depleted controls (77 ± 8 pmoles/g).

Pieces of thoracic cord from all animals were procured for CA fluorescence and the CA terminal patterns were compared. The most prominent effect of the A-11 lesions was a large unambiguous reduction in fluorescence in the DH. Regarding the possibility of a projection to the ILC, the present experiments were inconclusive.

The present experiments suggest that only part of the DA innervation of the spinal cord originates in the hypothalamic A-11 area; they provide more evidence in support of the suggestion (Skagerberg, Bjorklund, Lindvall & Schmidt, Br. Res. Bull., 9:237, 1982) that the A-11 group projects to the dorsal horn.

Supported by NIH HL 28785.

332.9 EVIDENCE FOR A STRONG, ORDERLY LOCUS COERULEUS PROJECTION TO RAT OLFACTORY BULB. M. T. Shipley+, F. R. Halloran and J. de la Torre. +Dept. of Anat. & Cell Biol., Univ. of Cincinnati, Col. of Med., Cincinnati, OH 45267 and Dept. of Physiol. & Neurobiol., Northwestern Univ., Chicago, IL 60201.

The locus coeruleus (LC) is currently one of the most fashionable structures in the brain. On a "per cell" basis, it may be the most intensively studied structure in the mammalian CNS. LC is located in the dorsolateral pons and, in the rat, contains approximately 1600 neurons (Swanson, Br. Res. '76). Strongly convergent evidence indicates that all of these neurons are noradrenergic; neuroanatomical studies show that LC is the major, and probably the sole source of noradrenergic innervation of the telencephalon. LC has been implicated in functions ranging from arousal, motivation, learning and sleep to a possible role in the development of functional cortical circuitry.

Following injections of WGA-HRP into the main olfactory bulb (MOB) we were surprised to find intense retrograde labeling of sizable proportion of ipsilateral LC neurons and a few contralateral ones as well. Injection sites comprising 25-50% of MOB labeled 450-600 LC neurons or about 30-40% of the total population; smaller injections labeled fewer cells. The number of LC neurons labeled was manifestly greater than following comparable injections in neocortical sensory fields (visual, auditory, somatic sensory, gustatory) or in insular, entorhinal or perirhinal cortex.

The organization of catecholamine (CA) fibers in MOB was studied by histofluorescence. A profuse network of CA fibers and terminals was found in the glomerular and internal granular layers. CA fluorescence in the external periform layer (EPL) was in general quite low but there was a strikingly regular pattern of single CA fibers traversing the EPL to enter single glomeruli. Two fibers entering a single glomerulus were never observed.

These results suggest that the MOB is one of the major targets of LC neurons in the rat. It would further appear that there may be a surprisingly orderly relationship whereby single LC axons innervate single olfactory glomeruli. This would suggest a hitherto unsuspected degree of specificity in an LC projection to the telencephalon.

How this exuberant, and possibly highly-ordered, LC-OB pathway fits into current theories of LC function is unclear. It would seem, though, that MOB is an ideal structure for investigating the organization, function, and development of this fascinating central noradrenergic pathway.

Supported by NIH NS 19730, NINCDS 18490 and USAMRDC DAMD 17-82-C-2272.

332.10 CATECHOLAMINE INNERVATION OF THE CAUDAL NEUROSECRETORY COMPLEX. K.E. Miller and R.M. Kriebel*. Department of Anatomy &Neurobiology, College of Medicine, Burlington, Vermont 05405.

Catecholamines have been implicated as neurotransmitters in the regulation or modulation of neuroendocrine cellular activity in many neuroendocrine systems. Our laboratory is involved in a multidisciplinary approach to the study of neurotransmitter induced postsynaptic responses in neuroendocrine neurons. The neuroendocrine neurons of the caudal neurosecretory complex (CNC) of Poecilia latipinna (green molly) is well suited for such an approach. It has been suggested based on ultrastructural examination that the neuroendocrine cells of the CNC receive catecholaminergic innervation. The catecholamine innervation of the CNC was examined using fluorescence microscopy. Ten animals were used for this study and were anesthetized with MS222. The terminal spinal cord segments were exposed and fixed in situ with 4% formaldehyde and 0.5% glutaraldehyde (FAGLU) in phosphate buffer, 0.1M, pH 7.0, 4°C. The tissue was left in FAGLU at 4°C for three hours and then embedded in polyethylene glycol(PEG). The tissue was sectioned at 10 microns, floated on warm FAGLU (40°C) and mounted on gelatin coated slides. Sections were examined and photographed with a Zeiss fluorescence microscope. After sections were examined, the tissue was counterstained with cresyl violet to verify the location of catecholamine fluorescence. Catecholamine fibers were observed within the neuroendocrine nucleus of the CNC. Cells within the nucleus exhibiting a yellow-orange autofluorescence were often apposed with catecholamine varicose fibers. These cells were identified as the neuroendocrine neurons of the CNC in cresyl violet counterstained sections. Along with catecholamine fibers found in the nucleus, a dense plexus of catecholamine varicosities was observed in the axonal tract of the neuroendocrine cells. In addition to catecholamine fibers, small intensely fluorescent cells were found in the CNC. These catecholamine containing cells were located in two areas. Groups consisting of three to seven cells were observed on the lateral edge of the neuroendocrine nucleus. Varicose fibers could occasionally be traced from these cells. Catecholamine cells were also found medially along the entire length of the CNC. Examination of counterstained sections revealed that these cells are intermingled with the cells of the CNC ependymal lining. This study indicates that the CNC receives a catecholaminergic innervation and establishes a base for further studies of catecholamine synaptic mechanisms among the neuroendocrine neurons of the caudal neurosecretory complex. Ultrastructural examination of the CNC is in progress to further understand the catecholamine innervation of the caudal neuroendocrine neurons.

(Supported by BNS-8206452).

332.11 X-RAY MICROANALYSIS OF DOPAMINE STORAGE SITES IN PRIMATE SUBSTANTIA NIGRA NEURONS, Joe Wood, B. Rosario*,P. Owens*. Dept. Neurobiology and Anatomy, Univ.of Texas Med. Sch., Houston, TX.77030.

The area compacta of substantiae nigra were dissected from the brains of stump-tail macaques and rhesus monkeys. All tissue was prepared for electron microscopy by fixation with glutaraldehyde in various buffers. In some blocks, the glutaraldehyde fixation was followed by a potassium dichromate (pH 4.1) treatment for dopamine localization. All tissues were embedded in epoxy resin; both thick, for light microscopy, and thin, for electron microscopy sections were made. Photographs were made at the light microscopic level, as well as, the electron microscopic level for point-point correlation. Selected areas of neurons were examined using the Kevex Energy Dispersive Analytical system on a JEOL 100 CX electron microscope. Characteristic inclusion bodies or organelles were identified and found to contain extraordinary amounts of chromium. At the same time in the same areas, analyses were taken for the determinations of calcium, sulfur, phosphorus, and arsenic. The organelles containing the largest amount of chromium indicative of high amounts of dopamine were also found to contain high values for arsenic, calcium, sulfur, and phosphorus, and are confined to a particular type neuron in the substantia nigra. Other neurons and organelles were less reactive, while still others were non-reactive. It is apparent that the particular neuron inclusions which are responsible for the packaging of the catecholamine can be distinguished by the methods described and since these organelles also possess characteristic ionic contents consistent with the chromium of dopamine storage, it is evident that electron density alone is not a sufficient discriminator of organelle content. Analyses of in vitro samples and model systems composed of glutaraldehyde, buffer and dopamine, indicate that arsenic is incorporated from the sodium cacodylate buffer, a certain amount of phosphorus can be incorporated from a phosphate buffer and small amounts of sulfur may be obtained from the tissue process of using sodium sulphate as an osmotic expander in the potassium dichromate solution. The various analyses of tissues prepared without the exogenous elements makes it now possible to develop a profile for dopamine storing organelles in the substantia nigra neurons. These neurons have been identified, both at the electron microscopic and light microscopic levels, and can be readily distinguished from other identifiable neuron-types in the substantia nigra. This work was supported in part by PHS Grant No. 10326.

332.12 CATECHOLAMINE CONCENTRATIONS IN SELECTED BRAIN REGIONS OF STRESS-SUSCEPTIBLE AND STRESS-RESISTANT PIGS. M. G. Erlander*, J. A. Parliman*, D. C. Beitz*, D. D. Draper*, and L. L. Christian*. (SPON: R. Netsell). Depts. of An. Sci., Biochem.-Biophys., and Vet. Anat., Iowa State Univ., Ames, Iowa 50011.

Concentrations of norepinephrine, dopamine, and epinephrine were measured in the frontal cortex, cerebellum, substantia nigra, and caudate nucleus of stress-susceptible and stress-resistant pigs. Eight stress-resistant pigs and twelve stress-susceptible pigs (90-100 kg body weight) were used in this study. The pigs were classified as stress-resistant and stress-susceptible by the combination of three methods: Halothane screening, H-blood typing, and blood creatine phosphokinase test. Concentrations of norepinephrine, dopamine, and epinephrine were measured by HPLC-electrochemical detection after an alumina extraction of homogenates of each brain sample. Concentrations (ng/mg of wet tissue) of norepinephrine were greater in cerebellum and caudate nucleus from stress-susceptible pigs than in same tissue from stress-resistant pigs. In contrast, concentration of norepinephrine was lower in substantia nigra from stress-susceptible pigs than in the same tissue from stress-resistant pigs. No differences, however, were found between concentrations of norepinephrine in the frontal cortex from both types of pigs. Dopamine concentrations were greater in the substantia nigra from stress-susceptible pigs than the same tissue from stress-resistant pigs. Individual catecholamine concentrations in different regions of the central nervous system of both types of pigs varied as follows: Norepinephrine -- cerebellum > frontal cortex > caudate nucleus > substantia nigra; dopamine -- caudate nucleus > substantia nigra > frontal cortex > cerebellum; epinephrine -- substantia nigra > caudate nucleus. Results of this study support our hypothesis that stress susceptibility in pigs is related to neurotransmitter metabolism in the central nervous system. Supported in part by American Parkinson's Disease Association.

332.PO DIFFERENTIAL TOXIC EFFECTS OF N-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (NMPTP) ON THE NIGROSTRIATAL AND MESOLIMBIC DOPAMINE SYSTEMS IN THE RHESUS MONKEY. R. S. Burns *, C. C. Chiueh, S. P. Markey*, M. H. Ebert*, D. M. Jacobowitz, and I. J. Kopin. Lab. of Clinical Science, NIMH, Bethesda, MD 20205.

Several young people developed a syndrome similar to Parkinson's disease (PD) after intravenous self-administration of illicit preparations of the synthetic analgesic 4-proprionoxy-4-phenyl-N-methylpiperidine which also contained a side product NMPTP (Davis, G. C., et al., Psych. Res. 1:249, 1979; Langston, J.W., et al., Science 219:979, 1983). IV administration of NMPTP to the rhesus monkey was shown to produce a similar neurological syndrome (akinesia, rigidity, postural tremor, flexed posture; reversed by L-dopa) and severe nerve cell loss in the substantia nigra (SN), pars compacta (Burns, R.S., et al., Proc. Natl. Acad. Sci.,in press, 1983.).

Monkeys given 5 doses (0.33 mg/kg) of NMPTP IV at 24 hr intervals and sacrificed at 1 month or 2 months after the last dose were compared with control animals. Tissue sections of the brain were stained for catecholamines using the glyoxylic acid method of histofluorescence. Tissue samples of specific brain nuclei were obtained by micropunch and the dopamine (DA) content determined by HPLC.

In the SN of the animals killed at 1 or 2 months an estimated 50% and 20%, respectively, of the normal number of flourescent A9 cell bodies were present with a relative preservation of the A10 cells. Very large numbers of swollen, distorted, intensely fluorescent DA-containing axons were seen in the area above the SN at 1 month with fewer seen after 2 months. The dense network of fluorescent nerve terminals in the nucleus accumbens (N.Acc.), olfactory tubercle (OT), and median eminence appeared normal at 1 to 2 months. The DA content of the caudate nucleus and putamen was less than 6% of the control value at both times, whereas the level of DA in the N.Acc. was unchanged.

NMPTP appears to destroy selectively neurons in the pars compacta of the SN (A8 and A9, nigrostriatal system) of the rhesus monkey leading to a marked reduction in the DA content of the striatum. DA terminals in the N.Acc. and OT originating from DA neurons in the ventral midbrain (A10, mesolimbic system) appear intact. The differential sensitivity of the nigrostriatal and mesolimbic DA systems to NMPTP suggests that a major biochemical difference exists between these two systems.

BIOGENIC AMINES II

333.1 CHARACTERIZATION OF A SEROTONIN RECEPTOR COUPLED TO ADENYLATE CYCLASE IN ADULT GUINEA PIG HIPPOCAMPUS. A. Shenker*, S. Maayani, H. Weinstein*, and J.P. Green. Department of Pharmacology, Mount Sinai School of Medicine of CUNY, New York, NY, 10029.

Serotonin (5-HT) stimulates adenylate cyclase activity by 90% in membranes from adult guinea pig hippocampus, apparently by acting on a distinct receptor (Shenker, et al., Life Sci. 32: 2335, 1983). Under the same assay conditions, 5-HT stimulated cyclase activity in hippocampal membranes from adult rats. The EC50 (0.1 uM) was the same in the two species, but maximal stimulation in rats averaged only 40% above basal activity. The relatively small response in adult rat membranes makes that system less suitable for quantitative pharmacological analysis. We have thus concentrated on characterizing the 5-HT response in guinea pig hippocampus by determining relative agonist potencies and affinities of competitive antagonists.

To examine the possibility that 5-HT cross-reacts at catecholamine receptors coupled to adenylate cyclase, we evaluated dopamine and l-isoproterenol as agonists in this system. Both are significantly less potent than 5-HT, and produced only 20% stimulation at a concentration of 10 uM. These findings, and results from additivity experiments indicate that 5-HT response is not due to activation of dopamine or beta-adrenergic receptors.

Concentration-response curves for 3 other indolealkylamines were obtained in this preparation; the maximal response was similar to that produced by 5-HT. 5-Methoxytryptamine was only slightly less potent than 5-HT (EC50 = 0.2 uM). The concentration-response curves for bufotenine (EC50 = 0.7 uM) and tryptamine (EC50 = 7 uM) were more shallow than that for 5-HT.

We evaluated the ability of drugs known to inhibit 5-HT responses in other systems to cause a dextral shift of the 5-HT concentration-response curve. The system was incubated with drugs for at least 8 minutes, and 5-HT stimulation was then assayed. 2-Bromo-LSD (BOL) and ketanserin appear to act as competitive antagonists of 5-HT-stimulated cyclase activity, with K_b values of 0.5 uM and 10 uM, respectively. BOL and ketanserin are 1000 and 10,000 times less potent as antagonists of 5-HT response in this system than as antagonists of 5-HT-induced contactile response in isolated rabbit aorta. This pronounced difference in apparent antagonist affinities suggests that the 5-HT receptor coupled to adenylate cyclase in guinea pig hippocampal membranes may be pharmacologically distinct from the vascular "D" 5-HT receptor (5-HT_2 site).

(Supported by UPHS grants DA-01875 and DA-00060 and NIH Medical Scientist Training Grant GM-07280 (A.S.))

333.2 SUBCELLULAR DISTRIBUTION OF SEROTONIN-1 RECEPTOR SUBTYPES. S. J. Waters* and D. L. Nelson. Dept. of Pharmacology & Toxicology, Col. of Pharmacy, Univ. of Arizona, Tucson, AZ 85721.

Recent studies have proposed the existence of at least two broad classes of serotonin (5-hydroxytryptamine, 5HT) receptors: the 5HT-1 and 5HT-2 receptors. Subsequent research has shown that the 5HT-1 receptor, which is defined by the high-affinity binding of ^{3}H-5HT, can be further subdivided into two groups based on their differential affinities for the neuroleptic spiperone. The present work extends these previous findings by examining the subcellular localization of the spiperone-sensitive ^{3}H-5HT binding sites in the rat cerebral cortex. Collection of subcellular fractions was performed using differential centrifugation. Four fractions were collected: the nuclear (N), the heavy mitochondrial (M), the light mitochondrial (L) and the microsomal (P) fractions. Saturation studies of the ^{3}H-5HT binding revealed no differences in the affinity of this ligand for its binding sites in any of the subcellular fractions. The number of binding sites differed between each fraction with fractions N, M, L and P containing 10%, 14%, 16% and 60% of the total tissue binding respectively. However, when binding was expressed on the basis of protein content there were no differences in any of the resulting Bmax values. Spiperone inhibition of ^{3}H-5HT binding revealed significant differences in the IC_{50} values between the subcellular fractions. Observed values were 7 nM in fractions N and M, 16 nM in fraction L and 114 nM in fraction P. A heterogeneity of sites was suggested from the finding that logit-log plots of the inhibition curves generated in each subcellular fraction produced slopes of less than 1.0. Nonlinear regression analysis with computerized curve fitting showed that the inhibition curves for each fraction fit a two-site model significantly better than a one-site model. The ^{3}H-5HT binding site having low affinity for spiperone gave Kd values which ranged from 1700-6000 nM. These dissociation constants are in agreement with those determined from prior binding studies using whole tissue homogenates. While both the high- and low-affinity sites were found in each fraction, their proportions were markedly different depending on the fraction examined. Fractions N, M, and L contained primarily the high-affinity site with the low-affinity site contributing only 17%, 10% and 16% to the total binding of each fraction respectively. In contrast, 33% of the binding sites were of the low affinity type in fraction P. Thus, there is a differential fractionation pattern of the sites having high and low affinity for spiperone which comprise the putative 5HT-1 receptor. These findings may be useful for future research by allowing isolation of a more homogeneous population of binding sites for pharmacological characterization. These data also indicate that caution should be used when comparing the potencies of compounds at the 5HT-1 binding sites, since these values may depend upon the subcellular membrane fraction which is being examined. (Supported by NIH Grant NS16605.)

333.3 SEROTONIN BINDING AND AGONIST PROPERTIES OF 8-HYDROXY-2-(N,N-DIPROPYLAMINO)TETRALIN (8-OH-DPAT). N. R. Mason*, R. D. Marsh*, K. W. Perry*, H. D. Snoddy* and R. W. Fuller. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285

Arvidsson et al (J. Med. Chem. 24: 921, 1981) have shown that 8-OH-DPAT is a direct-acting serotonin (5HT) receptor agonist as demonstrated by a decrease in brain 5-hydroxytryptophan (5HTP) formation and development of the "serotonin behavior syndrome". The compound had no effect on dopamine or noradrenaline receptor stimulatory properties. In contrast, the 5, 6 and 7 monohydroxy isomers were all active on dopamine systems but lacked 5HT activity. No in vitro receptor binding studies with 8-OH-DPAT were reported.

Rat brain cortex membranes were prepared according to the method of Nelson et al, and binding to serotonin receptors was done using the methods of Bennett and Snyder for $5HT_1$ and Peroutka and Snyder for $5HT_2$ receptors. Filtration of the membranes was done using Whatman GF/C filters on a Brandel M-24 cell harvester modified for receptor binding.

8-OH-DPAT (synthesized by R. Titus, Lilly Research Laboratories) competed with ^{3}H-5HT for binding, with an IC_{50} of 70 nM and a K_i value of 30 nM. Thus it has a slightly higher affinity than the serotonin agonist m-trifluoromethylphenylpiperazine but lower affinity than the antagonists methysergide or metergoline for the $5HT_1$ receptor. The Hill slope for the competition curve was 0.3, whereas that for 5HT was 0.9-1.0, suggesting that 8-OH-DPAT has different binding kinetics than does 5HT at $5HT_1$ receptors. 8-OH-DPAT inhibited ^{3}H-spiperone binding with an IC_{50} of 6000 nM, indicating it has little affinity for the $5HT_2$ receptor. The 5- and 6-OH or 6,7-diOH-DPAT isomers had little affinity for either the $5HT_1$ or $5HT_2$ receptor (IC_{50} values in the micromolar range).

8-OH-DPAT decreased 5HT turnover in rat brain in vivo. At s.c. doses of .01 mg/kg and higher, 8-OH-DPAT decreased 5HTP accumulation after decarboxylase inhibition in striatum and hypothalamus (confirming Arvidsson et al); higher doses (1-10 mg/kg) were required when the drug was injected i.p. 8-OH-DPAT lowered 5-hydroxyindoleacetic acid concentration in whole brain at doses of .01-1 mg/kg s.c., and at doses of .1-1 mg/kg s.c. caused a dose-related increase in serum corticosterone concentration in rats, these effects being characteristic of all other 5HT agonists we have studied.

These results confirm and extend the results of Arvidsson et al on the serotonin agonist properties of 8-OH-DPAT. In addition, receptor binding studies have shown it to have a preferential affinity for the $5HT_1$ receptor, although the binding kinetics are different than 5HT.

333.4 IN VIVO REGULATION OF ^{3}H-TRYPTAMINE BINDING SITES IN RAT BRAIN. Caren S. Cascio* and Kenneth J. Kellar (SPON: A. Raines). Department of Pharmacology, Georgetown University Schools of Medicine and Dentistry, Washington, DC 20007

^{3}H-Tryptamine (^{3}H-TA) binds to a site in rat brain which has characteristics of a tryptamine receptor (Kellar and Cascio, Eur. J. Pharmacol. 78, 1982). Tryptamine is a substrate for monoamine oxidase enzymes and treatment with a MAO inhibitor increases tryptamine levels in brain (Phillips and Boulton, J. Neurochem. 33, 1979). To determine whether tryptamine recognition sites are altered by a treatment which increases the brain concentration of tryptamine, rats were treated with the MAO inhibitor pargyline (10 mg/kg/day) for 12 days and tryptamine binding sites were measured in several brain areas. This treatment produced a 23-26% decrease in ^{3}H-TA binding in the cerebral cortex, striatum and hippocampus and a 44% decrease in the hypothalamus. Scatchard analyses in the cortex indicated a decreased density (B_{max}) of ^{3}H-TA binding sites but no change in affinity. Thus, chronic treatment with pargyline down-regulates ^{3}H-TA binding sites.

In rats treated chronically with reserpine (2.5 mg/kg, 2 days then 0.5 mg/kg every other day for 10 days), ^{3}H-TA binding was increased in the cortex (19%) and in the hippocampus (12%) but not in the striatum or hypothalamus. The increase in the cortex appears to be due to an increase in the B_{max} of ^{3}H-TA sites. The small increase in the number of ^{3}H-TA recognition sites following reserpine treatment and the variation across brain areas could be a reflection of varying rates of tryptamine synthesis in different brain areas. Nevertheless, the increase in ^{3}H-TA recognition sites following reserpine treatment suggests that brain tryptamine may be associated with reserpine-sensitive storage vesicles.

Chronic treatment with lithium which decreases the density of ^{3}H-serotonin receptor binding sites in the hippocampus, has no effect on ^{3}H-TA binding in the hippocampus, cortex or striatum. Electroconvulsive shock (once a day, 12 days) which increases the number of 5-HT-2 receptor binding sites labeled by ^{3}H-spiperone in the cortex, has no effect on ^{3}H-TA binding in cortex, striatum or hippocampus.

Lesions with 5,7-DHT or 6-OHDA do not alter ^{3}H-TA binding in the cortex, striatum, hippocampus or hypothalamus. Thus, the ^{3}H-TA recognition sites do not appear to be presynaptic on serotonin or catecholamine terminals.

The data indicate that ^{3}H-TA recognition sites can be distinguished from serotonin receptors on the bases of ligand specificity, in vitro pharmacology (Kellar and Cascio, 1982) and in vivo regulation.

333.5 USE OF GTP WHEN MEASURING AGONIST BINDING TO ^{3}H-5-HYDROXYTRYPTAMINE (^{3}H-5-HT) RECEPTOR SITES. M.A. Sills, B.B. Wolfe, and A. Frazer. Depts. of Pharmacology and Psychiatry, University of Pennsylvania and Veterans Administration Hosp., Phila., PA 19104.

Based on the finding of Pedigo et al. (J. Neurochem. 36:220, 1981) that spiroperidol displaced ^{3}H-serotonin (^{3}H-5HT) in a biphasic manner, it was suggested that there were two sub-types of the serotonin-1 receptor, designated 1A and 1B. However, ternary complex formation (i.e., multiple receptor states) can be involved in agonist binding; therefore, the effect of GTP on ^{3}H-5HT binding in rat frontal cortex was measured. In the absence of GTP, both saturation and competition binding curves were best fit by a two-component model, whereas in the presence of GTP (1mM) the data were no longer better fit by a two-component model, as revealed by non-linear regression analyses (MLAB). This is indicative of ternary complex formation and multiple states of the serotonin-1 receptor site. Even in the presence of GTP, spiroperidol displaced ^{3}H-5HT (15nM) in a biphasic manner, supporting the conclusion of Pedigo and associates of receptor sub-types. However, due to the presence of ternary complex, it is difficult to interpret displacement curves for serotonergic agonists in the absence of GTP; in particular, it cannot be determined whether serotonergic agonists have the same or different affinities for the two receptor sub-types. This is illustrated by our analysis of the displacement of 15nM ^{3}H-5HT by either bufotenin or d-lysergic acid diethylamide (LSD) in the absence or presence of GTP (1mM). Both of these drugs produced displacement curves fit significantly better by a two-component model than a one-component model ($p<0.001$) in the absence of GTP. When GTP was present, the LSD displacement curve was no longer fit significantly better by a two-component model. The IC50 for LSD displacement in the presence of GTP was 26±5nM. However, even in the presence of GTP, displacement of ^{3}H-5HT by bufotenin was still fit better by a two-component binding model ($p<0.005$) than by a one-component model. The IC50's of bufotenin for the high and low affinity sites were 70±37nM and 973±217nM, respectively. Since unlabelled serotonin, LSD and bufotenin all displaced the same amount of specific ^{3}H-serotonin binding, it appears that LSD binds with equal affinity to serotonin 1A and 1B receptor sites whereas bufotenin has different affinities for these sites. It seems necessary, therefore, to use GTP to eliminate ternary complex formation to assess whether serotonergic agonists have the same or different affinities for the sub-types of the serotonin-1 receptor. (Supported by Research Funds from the Vet. Admin. and USPHS Grants MH 29094, GM 07517 and GM 31155).

333.6 A RAPID, SENSITIVE PROCEDURE FOR ANALYSIS OF 2-PHENYLETHYLAMINE IN BRAIN TISSUE AND URINE. G.B. Baker, D.R. Hampson* and R.T. Coutts*. Neurochem. Res. Unit, Dept. of Psychiatry and Fac. of Pharmacy, Univ. of Alberta, Edmonton, Canada, T6G 2G3.

It has been proposed that 2-phenylethylamine (PEA), termed a trace amine because of its low absolute concentration in brain, is involved in the etiology of a number of psychiatric and neurologic disorders, including depression, schizophrenia and migraine. An electron-capture gas chromatographic procedure has been developed in our laboratories for quantitation of PEA in brain tissue and urine. Brain samples are homogenized in 5 volumes of ice-cold 0.1N perchloric acid and centrifuged at 12,000 x g for 15 min. Samples (4 ml) of this supernatant or of urine are made slightly basic with solid potassium bicarbonate, after addition of 2-(4-chlorophenyl)ethylamine as internal standard, and shaken with 4 ml of the liquid ion exchanger di-2-(ethylhexyl)phosphate (2.5% v/v in chloroform). Following centrifugation, the top layer is removed by aspiration. To the bottom layer is added 0.5 M HCl (2.5 ml). After shaking and centrifugation, the top layer is retained and made slightly basic by the addition of solid sodium bicarbonate. This is followed by acetylation using 300 µl of acetic anhydride (Martin and Baker, Biochem. Pharmac., 26, 1513, 1977). The solution is extracted with ethyl acetate (3 ml); this organic layer is washed by shaking briefly with water (300 µl). The top layer is retained and taken to dryness under a stream of nitrogen. To the residue is added 300 µl of toluene and 2 µl of pentafluorobenzoyl chloride. After heating at 80° for 30 min, the mixture is taken to dryness under a stream of nitrogen. The residue is taken up in toluene, and an aliquot is injected on a gas chromatograph equipped with an electron-capture detector and a fused silica capillary colum (OV-101, 10 m). The oven temperature is maintained at 105° for 0.5 min and is increased at a rate of 25°/min to 270°. The retention time of the derivatized PEA under these conditions is 6.6 min and that of the derivatized internal standard is 7.5 min.

Funded by the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research.

333.7 OCTOPAMINE: A NEUROHORMONE WITH PRE-SYNAPTIC EFFECTS AUGMENTED BY ACTIVITY. C. A. Breen* and H. L. Atwood. Dents. of Zoology and Physiology, Univ. of Toronto, Toronto, Ontario, M5S 1A8.

Octopamine, the phenol analogue of norepinephrine, at concentrations as low as 10^{-11} to 10^{-10}M caused an increase in the amount of tension generated in the crayfish opener muscle, which was accompanied by a striking increase in the amplitude of excitatory post-synaptic potentials (EPSP's). EPSP amplitudes increased up to 100% in a dose-dependent manner over the range of 10^{-10} to 10^{-8}M octopamine; such enhancement could not be accounted for by changes in muscle fiber input resistance, which were at most 16%.

Experiments in which the quantal output of individual nerve terminals was monitored showed that 10^{-10}M octopamine caused a substantial increase in quantal content, which was parallel with observed increases in EPSP amplitude. Octopamine appeared to act presynaptically to increase transmitter release in much the same manner as serotonin (Dudel, J., Nauyn-Schmiedebergs Arch. exp. Path. u Pharmak., 249:515-528, 1965).

Both the magnitude and the duration of EPSP amplitude and quantal content enhancement were augmented by activity. A 10 sec train of stimuli at 15 Hz delivered every second minute was used in most experiments, as octopamine is thought to be released by neurosecretory cells during stressful of active periods. A less active regime of one train every 20 minutes approximates a resting state, and was used to compare the efficacy of octopamine in "active" and "inactive" preparations. Octopamine was almost twice as effective in neurally "active" preparations than in "inactive" preparations, suggesting that octopamine may function to promote transmission selectively at synapses during periods of prolonged stress to the animal, or during particular locomotor activities.

Supported by grants from MRC and NSERC to H. L. Atwood.

333.8 MORPHOLOGY AND PHYSIOLOGY OF THE NON-SEROTONERGIC DORSAL RAPHE PROJECTION NEURONS. M. R. Park, Dept. of Anatomy, Mich. State Univ., E. Lansing, MI 48824.

Intracellular recordings using horseradish peroxidase (HRP) filled microelectrodes were made among those neurons in the rat dorsal raphe (DR) nucleus which exhibit a rapid rate of spontaneous firing. Evidence from a variety of sources has suggested that these cells are neither serotonergic nor projecting and correspond to the class of small neurons consistently reported, but variously described in studies using the Golgi method. The cells labeled with intracellular HRP do have small (10-15 μm) round somata. Somatic spines, if present, are few in number but can be elaborate, appearing as filiform processes up to 10 μm in length. The dendrites are characteristically fine (ca. 0.3 μm) and highly beaded. They may be as long as 0.5 mm. Like the medium-sized serotonergic DR neurons, the dendrites are radiating and poorly branching. Distinct from these, however, each small cell usually has one or two dendrites of more tufted appearance, where a short primary dendrite divides into a total of 3-6 secondary and tertiary branches. Dendrites end either as a single strand or as a simple thicket of just three or four terminating branches.

The axons of small DR neurons project from the nucleus. A main axon arises from the soma or a primary dendrite and shortly tapers to about 0.5 μm in diameter. It maintains this diameter, with few varicosities, as it takes an indeterminate course while within the dendritic domain of the parent cell. Before leaving the vicinity of the cell, it becomes oriented along the sagittal rostro-ventral diagonal taken by the axons of most DR projection neurons and leaves the nucleus. During its intranuclear course, the main axon gives off numerous fine (ca. 0.3 μm) and highly beaded collaterals. Some of these contribute to a plexus within the neighborhood of the parent cell while others radiate for 100-500 μm in a straight path which does not respect nuclear boundaries.

Stimulation of ventral mesencephalic tegmentum elicits a short-latency antidromic action potential and an inhibitory postsynaptic potential which produces a pause (ca. 20 ms) in the spontaneous firing of these cells. No response to stimulation of lateral habenula or the fasciculus retroflexus has been observed. Action potential shape is distinct from that of serotonergic DR neurons: The rising and falling limbs of the action potential are equally fast and the spike monotonically repolarizes to a sharp and brief after-hyperpolarization.

(Supported by NIH Grant NS 19079. Recordings were made using the facilities of Dr. S. T. Kitai).

333.9 ELECTROPHYSIOLOGICAL INVESTIGATION OF HISTAMINE-GABA INTERACTIONS IN THE RAT, S. A. Springfield*, A. Tiberio* and H. M. Geller, Dept. of Pharmacology, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854

The role of histamine (HA) as a putative neurotransmitter or neuromodulator has become increasingly important. In this series of experiments we have examined the hypothesis that HA modulates the strength of local GABAergic inhibitory neurotransmission in the hippocampus, as well as the possibility that HA modulates the rat cerebral cortex in situ.

The hippocampal experiments were performed on 300-350 μm thick transverse slices prepared from 125-200 g male rats. The slices were constantly superfused with a bicarbonate-buffered balanced salt solution equilibrated with 95% O_2/5% CO_2 and maintained at 34-35° C. A bipolar stimulation electrode was placed in stratum radiatum at the border of CA1-CA2, and paired stimuli (2-20 V) were delivered at 1/60 HZ. Evoked population spikes were recorded with a single barreled glass microelectrode in stratum pyramidale of area CA1. Under these circumstances, the amplitude of the test spike differs from that of the conditioning spikes; the ratio is a function of the interstimulus interval: shorter intervals produce "paired pulse" inhibition whereas longer intervals produce facilitation. These events have been attributed to the action of a GABA-mediated recurrent inhibition superimposed upon orthodromic excitation.

HA (10^{-6}-10^{-5}M) added to the superfusate generally resulted in an increase in amplitude (10-40%) of the test response at any given interval as compared to control responses. In some cases the increase was accompanied by the appearance of secondary and tertiary population spikes. On rare occasions HA produced a reduction of the test response. No consistent change was observed in the amplitude of the conditioning response during the perfusion of HA.

In rat cerebral cortex in situ, the firing of spontaneously active sensorimotor cortical neurons was recorded with 7-barreled microelectrodes. Cortical neuronal activity was reproducibly slowed by the iontophoretic application of GABA (5-10 nA, 5-20 sec pulses). Concomitant continuous application of HA (up to 10 nA) reversibly reduced the depressant activity of GABA (16-58%) while variably reducing spontaneous activity. Higher HA ejection currents markedly reduced spontaneous activity, obscuring any actions of GABA.

In summary, our data demonstrate a diminution of the efficacy of GABA by HA in rat brain, thus supporting a modulatory role for this amine in regulating neuronal excitability.

Supported by NIH grants NS-15468 and NS-19187 as well as an NIH Postdoctoral Fellowship #NS-07119 to S.A.S.

333.10 ELECTRICAL STIMULATION OF THE DORSAL RAPHE NUCLEUS INCREASES 5-HYDROXYTRYPTOPHAN ACCUMULATION IN BRAIN REGIONS CONTAINING SEROTONERGIC NERVE TERMINALS. N.J. Duda and K.E. Moore. Dept. of Pharmacol./Toxicol., Michigan State Univ., East Lansing, MI 48824.

Following administration of an aromatic L-amino acid decarboxylase inhibitor, dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5HTP) accumulate in brain regions containing dopamine (DA) and 5-hydroxytryptamine (5HT) nerve terminals, respectively. The rate of accumulation of these precursors has been used as a biochemical index of DA and 5HT neuronal activity (Carlsson et al., N.S. Arch. Pharmacol. 275: 153, 1972). Electrical stimulation of DA neurons increases the rate of DOPA accumulation in forebrain regions containing terminals of these neurons (Murrin and Roth, Mol. Pharmacol. 12: 463, 1976) and results of a preliminary report (Boadle-Biber et al., Biochem. Pharmacol. 32: 185, 1983) suggest an increased rate of 5HTP accumulation in cerebral cortex following electrical stimulation of 5HT neurons in the dorsal raphe. The purpose of the present study was to extend the latter findings by measuring concurrently the rate of accumulation of DOPA and 5HTP in selected brain regions of rats treated with a decarboxylase inhibitor (NSD 1015).

Male Sprague-Dawley rats anesthetized with chloral hydrate (400 mg/kg, i.p.) were sacrificed 0, 10, 20 or 30 minutes following NSD 1015 administration (25 mg/kg, i.v.). High performance liquid chromatography with electrochemical detection was used to measure the concentrations of DOPA and 5HTP in nucleus accumbens, striatum, septum and mediobasal hypothalamus. In each region DOPA and 5HTP accumulated linearly with time through 30 minutes. A bipolar stainless steel electrode was positioned in the dorsal raphe nucleus and NSD 1015 was injected concurrent with the onset of electrical stimulation of the nucleus (monophasic pulses, 0.3 mA, 10 Hz, 1 msec duration). Thirty minutes of stimulation had no effect on the accumulation of DOPA in the nucleus accumbens, striatum or septum, but it did produce a small increase in DOPA accumulation in the mediobasal hypothalamus. In contrast, a marked increase in 5HTP accumulation was elicited in the nucleus accumbens, striatum and septum, and a somewhat lesser increase was observed in the mediobasal hypothalamus. As expected, stimulation did not change the accumulation of 5HTP in the thoracic spinal cord, a region which does not receive projections from the dorsal raphe nucleus. These results indicate that 5HTP accumulation following decarboxylase inhibition is a valid index of 5HT neuronal activity. (Supported by NIH grant NS15911.)

333.11 SEROTONIN INDUCED DEPOLARIZATION OF SPINAL MOTONEURONES FOLLOWING BLOCKADE OF SYNAPTIC TRANSMISSION. R.S. Neuman, Faculty of Medicine, Memorial University, St. John's, Newfoundland, Canada A1B 3V6.

The ventral horn of the lumbar spinal cord in vertebrates receives an extensive terminal plexus of serotonin (5HT) containing fibres from perikarya located in the paramedian region of the caudal brainstem. Application of 5HT receptor agonists to lumbar spinal motoneurones facilitates glutamate evoked firing of these neurones, an action thought to result from depolarization of the motoneurone (Neuman and White, Eur. J. Pharmacol. 81:49-56; 1982). It has been suggested that this excitatory action of 5HT on spinal motoneurones might result from inhibition of local interneurones. This hypothesis was tested in vitro using hemisected spinal cords from frogs and immature rats (7 to 11 days old). Polarization of the motoneurones and evoked synaptic activity were recorded differentially between the spinal cord and the cut end of a ventral root electrically isolated from the cord by vaseline or parafin oil.

5HT (1 nM to 0.1 mM) depolarized both rat and frog motoneurones in a dose dependent manner thus confirming previous reports. The response to 5HT was similar in rat and frog motoneurones, a slow rising phase followed by a very slow return to baseline. Compared to the rat however, there was considerable variability in the sensitivity of frog motoneurones to 5HT.

To determine whether 5HT was acting indirectly on motoneurones via changes in synaptic activity of interneurones, synaptic transmission was blocked either by elevating Mg^{2+} concentration (20 mM) or lowering Ca^{2+} (0.38 mM) concentration and raising Mg^{2+} concentration (10 mM) of the perfusion media. The effectiveness of these procedures was demonstrated by the gradual failure to evoke ventral root responses with dorsal root stimulation. Following cessation of evoked activity, 5HT remained effective in depolarizing both rat and frog motoneurones. The change in Ca^{2+} and/or Mg^{2+} concentrations reduced the amplitude of the 5HT response to some extent as it did the response to glutamate (1 mM). The form of the response to 5HT remained the same however. The ability of 5HT to depolarize spinal motoneurones in the absence of synaptic activity suggests that 5HT has a direct action on these neurones.

Supported by the MRC (Canada)

333.12 INCREASE IN TRYPTOPHAN HYDROXYLASE (TrpH) ACTIVITY BY STIMULATION OF CENTRAL SEROTONERGIC NEURONS. M.C. Boadle-Biber, J.N. Johannessen*, N. Narasimhachari and T-H. Phan*, Dept. of Physiol. Med. Coll. of Virginia, Richmond, VA 23298.

Others have shown that electrical stimulation of serotonergic neurons in the raphe nuclei of the midbrain enhances the in vivo formation of serotonin (5-HT) in the terminal projections of these neurons, through an increase in the conversion of tryptophan (trp) to 5-hydroxytryptophan (5-HTP) by TrpH, the rate limiting enzyme in 5-HT synthesis. We recently found that in vivo electrical stimulation of rat dorsal raphe (DRN) increases the in vitro activity of TrpH in low speed supernatant fractions from cerebral cortex (1), thus suggesting that an alteration in the kinetic properties of TrpH may mediate the in vivo increase in 5-HT synthesis in response to stimulation. We now report that this increase in enzyme activity is sensitive both to duration and frequency of stimulation and is fully reversible. In these experiments stimulating electrodes (size 00 insect pins) insulated to within 0.5 mm of the tip were positioned stereotaxically in the DRN of chloral hydrate (400 mg/kg i.p.) anesthetized male Sprague Dawley rats, grounded with a rectal probe. Stimulation consisted of constant current monopolar square waves (200 uamps, 1.5 msec pulse width and 2-20 Hz). Experimental animals received continuous stimulation of 5-30 min duration, while shams were unstimulated. The effectiveness of the stimulation was monitored either by measuring the increase in brain tissue levels of the 5-HT metabolite, 5-hydroxyindoleacetic acid, or of 5-HTP that accumulates after pretreatment with an aromatic amino acid decarboxylase inhibitor (Ro4-4602, N^1-DL-seryl-N^2-(2,3,4-trihydroxybenzyl)-hydrazine, 800 mg/kg i.p.). After stimulation, animals were killed by decapitation and cerebral cortices, caudate nuclei and hippocampi rapidly frozen prior to assay for tissue 5-hydoxyindoles or for in vitro TrpH activity in low speed supernatant extracts incubated with 200 uM L-trp and 50 uM artificial reduced pterin cofactor, 6-methyl-5,6,7,8-tetrahydropterin. 5-hydroxyindoles were quantitated by high performance liquid chromatography with electrochemical detection (1,2). Brain regions innervated by DRN (caudate and cortex) showed an increase in TrpH activity with DRN stimulation (15 Hz, 20 min) while those receiving little or no input (hippocampus) showed no change in enzyme activity. Cortical enzyme activity was maximal after 20 min stimulation at 10 Hz. When frequency was varied with 20 min stimulation, enzyme activity was highest at 15 Hz and returned to control levels 30 min after stimulation ended. Supported by grant #NS 14090 from NINCDS. 1. Biochem. Pharmacol. 32, 185 (1983) 2. Res. Comm. Chem. Pathol. Pharmacol. 37, 413 (1982).

333.13 DENERVATION SUPERSENSITIVITY TO SEROTONIN IN THE RAT SPINAL CORD IS NOT DUE TO THE ABSENCE OF SEROTONIN. L.E. Tremblay*, P.J. Bédard, R. Maheux* and T. DiPaolo. Lab. Neurobiologie, Dept d'Anatomie, and Centre de Recherche en Endocrinologie moléculaire, Univ. Laval, Qué. G1J 1Z4.

We have previously established (Neuropharmacology 20, 611-616, 1981) that in rats, pretreated twenty one days before with the neurotoxin 5-7 DHT, lumbar neurons become supersensitive to 5-HT agonists as shown by increased EMG response in their hindlimbs 24 hours after spinalization. The present experiment was devised to study whether a similar depletion of 5-HT in the spinal cord caused by synthesis inhibition would also cause supersensitivity.

In a first experiment, three groups of ten rats received one of the following treatment: (1) PCPA 400 mg/kg or saline every third day for twenty one days; (2) 5-7 DHT 200µg intrathecally through a permanent cannula inserted through the cisterna magna and lowered to the lumbar subarachnoid space. After twenty one days, all animals were spinalized and twenty four hours later, received quipazine 10 mg/kg. Their motor response in the hindlimbs was measured as the spontaneous EMG activity (integrated) following the injection as compared to the baseline EMG level. After the physiological test, all animals were sacrificed, their spinal cord removed and the lumbo-sacral segment was assayed for 5-HT and 5-HIAA with H.P.L.C. The 5-HT level was decreased by 99.6% in the PCPA group and by 95% in the 5-7 DHT group as compared to controls (14.2 ng/mg of protein).

EMG responses to quipazine (average for twenty minutes) were 527% (controls) 240%,(PCPA) ($P<0.05$) and 421% (5-7DHT). A similar experiment with 5-7DHT was devised using a smaller dose of quipazine (1 mg/kg) in which the control animals also had a permanent cannula inserted and received the vehicle instead of 5-7DHT. Average EMG responses to quipazine were 204% (5-7DHT) and 115% (controls) ($P<0.05$). The response to 5-HTP 100 mg/kg i.p. was also measured twenty four hours later and was found to be 383% (5-7DHT) and 101% (controls) ($P<0.01$).

We therefore conclude that, denervation with 5-7DHT causes supersensitivity to small doses of quipazine and even more to 5-HTP. Comparable depletion of serotonin by synthesis inhibition is not followed by supersensitivity but rather by a decreased response to quipazine.

Our data suggest that, the trigger of supersensitivity in the serotonin system is not the absence of the neurotransmitter alone but rather the absence of the terminals or perhaps some other substance contained in these terminals.

333.14 SEROTONIN-2 RECEPTOR SUBSENSITIVITY IN RAT CEREBRAL CORTEX AFTER SUBCHRONIC (21 DAY) TREATMENT WITH VARIOUS NEUROLEPTIC AGENTS. Masahiko Mikuni#, Terrance H. Andree# and Herbert Y. Meltzer (SPON: M. Lowy). Dept. of Psychiatry, Univ. of Chicago Pritzker Schl. of Med., Chicago, IL. 60637.

Several studies have indicated that prolonged treatment with antidepressants reduce the density of 5-HT_2 receptor sites labeled by ^{3}H-spiroperidol in cerebral cortex, whereas Peroutka and Snyder (1980) reported that subchronic (21 day) treatment with either chlorpromazine (CPZ) or haloperidol (HAL) had no effect on these receptors. However, it is well known that the affinities of neuroleptics for 5-HT_2 receptors are similar to those of antidepressant drugs and that neuroleptics are equally potent antagonists of the 5-hydroxytryptophan-induced syndrome in rats. In addition, there is significant evidence from controlled clinical studies that certain neuroleptic drugs alone, or in combination with classical antidepressants, ameliorate the symptoms in subtypes of depression refractory to antidepressants alone.

Adult male Sprague Dawley rats (170 g) were treated with CPZ (10 mg/kg), perphenazine (1 mg/kg), thioridazine (10 mg/kg), promethazine (5 mg/kg), HAL (1 mg/kg), spiroperidol (0.5 mg/kg), moperone (1 mg/kg), cis-flupentixol (1 mg/kg), sulpiride (10 mg/kg) or an equivalent volume of saline. All animals were sacrificed 24 hours after the last injection and the brains removed, dissected on ice and stored at -70° C until assayed. Membranes of cerebral cortex were incubated with ^{3}H-Spiroperidol (0.16-2.30 nM) at 37° C for 20 min in 50 mM Tris-HCl (pH 7.4 at 25° C). Cinanserin (10 µM) was used to determine specific binding.

CPZ (60%), perphenazine (13%), thioridazine (21%), promethazine (33%), spiroperidol (26%), moperone (16%) and cis-flupentixol (30%) decreased the density (Bmax) of 5-HT_2 receptors, as shown in parentheses, compared to saline controls. HAL and sulpiride were without effect. In another study (14 day treatment) CPZ (10 mg/kg), imipramine (20 mg/kg) and CPZ + imip. decreased the density of 5-HT_2 receptors by 34, 22 and 51%, respectively. It is noteworthy that CPZ potentiated, over two-fold, the decrease in Bmax seen by imipramine alone. None of the drugs tested altered the K_D values obtained. In addition, single injections of CPZ and imipramine produced no changes in 5-HT_2 binding.

These results indicate that various classes of neuroleptics, as well as the classical antidepressants, are capable of inducing 5-HT_2 receptor down regulation.

These results, plus earlier results showing that neuroleptic treatment had no effect on α_2 or beta-adrenergic receptor binding suggests that the effects of neuroleptic drugs on cortical 5-HT_2 receptors may be related to their ability to improve the outcome of antidepressant treatment in certain subtypes of depression.

333.15 DO D-AMPHETAMINE (AMP) AND D,L-2,5-DIMETHOXY-4-METHYLAMPHETAMINE (STP) ACT ON SEROTONERGIC OR ADRENERGIC RECEPTORS ON HIPPOCAMPAL CA_1 CELLS *IN VIVO*? N.J. Penington* and R.J. Reiffenstein, Dept. of Pharmacology, University of Alberta, Edmonton, Alta. Canada. T6G 2H7.

The phenylisopropylamine 2,5-dimethoxy-4-methylamphetamine (STP) has a similar structure to mescaline but is approximately 50-100 times more potent as a hallucinogen. STP has been reported to release serotonin (5HT) and catecholamines, as has d-amphetamine, and alternatively, to directly stimulate post-synaptic receptors.

Male Sprague Dawley rats were anaesthetized with urethane 1.25 g/kg i.p., and in some cases supplemented with 100 mg/kg chloral hydrate i.v. Firing of hippocampal CA_1 cells was recorded using five-barreled electrodes. Cell firing was maintained by iontophoretic acetylcholine or glutamate application. Blood pressure was monitored in all experiments.

Antagonism of 5HT was attempted with cyproheptadine (n=5) and methysergide (n=9) (as reported by Segal: Brain Res. 103, 161, 1976) and with metergoline (n=4). No blockade of 5HT-induced inhibition was observed, even at doses of antagonists which themselves depressed firing. However post-inhibition (rebound) excitation was reduced. (No rebound excitation occurred after STP or AMP). In eight experiments a possible interaction between iontophoretically applied serotonin (5HT) and STP was examined. No evidence of blockade, occlusion or desensitization of responses was seen.

Effects of AMP and STP were compared, and the effects of α and β antagonists (phentolamine and sotalol) tested. Both STP and D-amphetamine caused a gradual decrease in firing rate of the CA_1 units at a low iontophoretic current (15 nA). Sotalol did not alter submaximal inhibitory responses to amphetamine (n=4) or STP (n=5), even at doses which caused the CA_1 firing to decrease. Phentolamine also did not interfere with the responses of D-amphetamine (n=4) or STP (n=4) and caused a noticable decrease in firing rate without altering spike height.

I.v. STP (250 ug/kg) and D-amphetamine (0.5 mg/kg) caused rises in blood pressure which exactly paralleled the rise in CA_1 firing rate; methacholine 10 ug/kg caused a brief fall in blood pressure which also was accompanied by a brief fall in firing rate. Thus results of i.v. experiments must be interpreted with caution, and fluctuations in blood pressure may alter responsiveness of CA_1 cells.

Evidence for interactions of AMP and STP with serotonergic receptors in CA_1 is inconclusive, but these experiments suggest neither interacts directly or indirectly with adrenergic transmission in CA_1.

N.P. holds a studentship of the AHFMR.

333.16 INTRAVENTRICULAR INJECTIONS OF ANTIBODY FOR MELATONIN AND CORTICAL EEG IN THE BEHAVING RAT. J. H. Peck, J. Stevens*, L. J. Grota, and G. N. Brown. Dept. of Psychology, Ithaca College, Ithaca, NY 14850.

Last year we reported on the effects of antibody for melatonin on anesthetized rats (Pierce, M. et al. Neurosci. Abst. 8, 1982). Unfortunately, neither pentobarbital nor urethane provided a stable enough baseline EEG with which to compare the intraventricular antibody injection. As we reported last year, a number of investigators have shown behavioral and EEG effects of melatonin in a variety of species, including improvement of symptoms in temporal lobe epilepsy patients. It is conceivable that injections of antibody for melatonin might produce cortical epileptoform activity. The purpose of this study was to examine this hypothesis in the behaving rat which would eliminate previous baseline problems due to anesthetic.

Male Charles River rats (CD) were anesthetized with Napentobarbital and had a guide cannula placed 1 mm above the lateral ventrical. Two pairs of bone screws placed ipsi and contralateral to the cannula acted as surface electrodes. Following recovery from surgery, the rats were placed in a faraday cage where they were connected to a swivel and baseline recordings were taken for 30 min. Next, an injection cannula was placed into the guide cannula and either 10 ul of antibody for melatonin or artificial CSF was injected at the rate of 1 ul/min. Recordings were made for an additional 30 min. and the records were examined for evidence of high voltage spiking.

Although some rats still show spiking, the baseline recording of the behaving rats was much more stable than the anesthetized rats. While the antibody for melatonin did not produce spiking in all cases, there appears to be an effect of the injection over that of the control injection. Recordings will be presented which show the range of results obtained.

333.17 REGIONAL ANALYSIS OF BIOGENIC AMINE TURNOVER IN RAT BRAIN AFTER EXPOSURE TO ELECTRICALLY CHARGED AIR MOLECULES (AIR IONS). W. H. Bailey, J. M. Charry*, J. M. Weiss, K. Cardle* & M. Shapiro* Laboratory of Behavioral Biology, The Rockefeller University, New York, NY 10021

It has been reported that exposure of mice or rats to high concentrations of positively or negatively charged air ions reduces the concentration of serotonin (5HT) in brain (Krueger & Kotaka, 1969; Diamond et al., 1980). We attempted to replicate these findings and also to determine if exposure to air ions affects the turnover of norepinephrine (NE) and dopamine (DA).

Male Holtzman rats were placed in custom built environmental chambers (Charry et al., submitted) and exposed for 18 hours to either positively or negatively charged air ions produced by corona discharge at a concentration of 5.0×10^5 ions/cm^3. Rats exposed to ambient levels of air ions (300 ions/cm^3) or to d.c. electric fields of similar polarity and strength (3.0 kV/m) served as experimental controls. The data was accumulated for 15 rats per condition. Biochemical analyses were conducted on an automated dual-column HPLC fitted with electrochemical detectors.

Exposure to positive or negative ions did not affect the concentration of 5HT, NE, or DA in individual brain regions including the striatum, hypothalamus, hippocampus+amygdala, brainstem, and frontal cortex. Estimates of catecholamine turnover as provided by the concentration of NE and DA in these brain regions measured two hours after the injection of 250 mg/kg of alpha-methyl-p-tyrosine were also not affected by exposure to air ions. The turnover of 5HT as indexed by the concentration of 5-hydroxyindoleacetic acid (5HIAA), or the ratio of 5HIAA/5HT was likewise not affected by ion exposure.

We conclude from these experiments that an exposure of Holtzman-strain rats for 18 hours to a high concentration of charged air ions does not significantly affect the concentration or release of NE, DA, or 5HT in brain.

(Supported by grants from EPRI)

333.18 EFFECT OF HYPOPHYSECTOMY AND SUBSEQUENT PROLACTIN ADMINISTRATION ON HYPOTHALAMIC 5-HYDROXYTRYPTAMINE IN OVARIECTOMIZED RATS. T.S. King, W.W. Morgan and R.W. Steger*. Depts. of Anatomy and OB/GYN, The Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX 78284.

Hyperprolactinemia whether of natural or experimentally-induced cause results in an increased turnover of the prolactin (PRL)-inhibiting factor dopamine within tuberoinfundibular neurons, suggesting that PRL can regulate its own release via a short-loop feedback mechanism. Although it is known that activation of the hypothalamic 5-hydroxytryptamine (5HT) system stimulates PRL release, the possibility that PRL could alter metabolism or release of this amine within the hypothalamus has not been examined. Bilaterally ovariectomized adult Sprague-Dawley rats were divided into three experimental groups: (1) hypophysectomized rats (HYPOX), (2) hypophysectomized rats injected subcutaneously with 0.5 $mg \cdot kg^{-1}$ of ovine PRL at 1000h and 1800h on the day prior to killing the rats (HYPOX + PRL) and (3) rats without further surgical manipulation (control). Three weeks after surgical manipulation, the rats were injected intravenously with 100 $mg \cdot kg^{-1}$ of NSD-1015 (3-hydroxybenzylhydrazine) and killed 15 or 30 min later. 5-Hydroxytryptophan and 5HT were measured by high performance liquid chromatography with electrochemical detection. 5-Hydroxytryptamine content was increased in the median eminence (ME) (2.56 ± 0.11 $ng \cdot ME^{-1}$) and mediobasal hypothalamus (MBH) (4.56 ± 0.43 $ng \cdot mg\ MBH^{-1}$) but not anterior hypothalamus (AH) (3.82 ± 0.69 $ng \cdot mg\ AH^{-1}$) of HYPOX rats in comparison to the 5HT content of these areas (1.49 ± 0.26 $ng \cdot ME^{-1}$, 3.07 ± 0.55 $ng \cdot mg\ MBH^{-1}$ and 3.01 ± 0.70 $ng \cdot mg\ AH^{-1}$, respectively) in control rats. This effect on 5HT content was reversed in the median eminence and mediobasal hypothalamus of HYPOX + PRL rats. Likewise, the rate of 5HT synthesis estimated by the linear increase in 5-hydroxytryptophan levels following NSD-1015 injection was increased in the median eminence (0.03 $ng \cdot ME^{-1} \cdot min^{-1}$) and mediobasal hypothalamus (0.079 $ng \cdot mg\ MBH^{-1} \cdot min^{-1}$) but not anterior hypothalamus (0.039 $ng \cdot mg\ AH^{-1} \cdot min^{-1}$) of HYPOX rats in comparison to the rate of synthesis of this amine in these areas (0.014 $ng \cdot ME^{-1} \cdot min^{-1}$, 0.039 $ng \cdot mg\ MBH^{-1} \cdot min$ and 0.039 $ng \cdot mg\ AH^{-1} \cdot min^{-1}$, respectively) of control rats. This effect was also reversed in the median eminence and mediobasal hypothalamus of HYPOX + PRL rats. These observations depict an inverse relationship between the level of circulating PRL and 5HT metabolism in the median eminence/mediobasal hypothalamus of ovariectomized rats, suggesting that hypothalamic 5HT stimulation of PRL release may involve a reciprocal, short-loop feedback mechanism. (Supported by NIH grant RR 07187 to TSK, NIH grant DA 00755 and Research Scientist Development Award DA 00083 to WWM and NIH grant HD 10202 [Neuroendocrine core].)

333.19 THE ACUTE EFFECTS OF METHYLPHENIDATE ON THE SEROTONERGIC SYSTEM OF THE RAT BRAIN. M. A. Peat, P. F. Warren* and J. W. Gibb, Dept. of Biochem. Pharmacol. & Toxicol., University of Utah, Salt Lake City, UT 84112.

Methylphenidate increases dopamine release and inhibits reuptake in the neostriatum. There are no published data concerning the effect of the drug on the serotonergic system of the rat brain. This report describes the acute effects of methylphenidate on this neurotransmitter system. Male Sprague-Dawley rats (200-300 g) were administered methylphenidate (50 or 100 mg/kg, i.p.) and sacrificed by decapitation 3 or 6 hours later. Cerebral cortex, neostriatum and hippocampus were dissected out and stored at -70°C before analysis. Tryptophan hydroxylase (TPH) activity was measured by a $^{14}CO_2$ trapping procedure (Life Sci., 25:1373, 1979) and serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and tryptophan (TRP) concentration by HPLC-fluorescence (Anal. Biochem. 128:275, 1983).

Cortical TPH activity was reduced to 74 and 68% of control 6 hours after an acute injection of 50 and 100 mg/kg respectively; after 3 hours no significant differences were seen in enzyme activity. Cortical 5-HIAA and TRP concentrations were increased significantly over control by both doses at 3 hours, but had recovered by 6 hours after injection of 50 mg/kg. Serotonin concentrations were unchanged by either dose. Hippocampal and neostriatal TPH activity were not significantly different from controls after either dose at 3 or 6 hours. Neostriatal tyrosine hydroxylase was decreased to 72% of control 6 hours after injection of methylphenidate (100 mg/kg).

These data demonstrate that methylphenidate selectively decreases cortical TPH activity. This could be due to increased release of 5-HT, as reflected by increased concentrations of 5-HIAA, following administration of high doses of the stimulant.

Supported by USPHS Grant DA-00869 and The Thrasher Research Fund.

333.20 HALLUCINOGENIC DRUG INTERACTIONS WITH S_1 AND S_2 CORTICAL SEROTONIN RECEPTORS. G. Battaglia, M. Shannon*, R.A. Glennon* and M. Titeler. University of Toronto, Toronto, Ontario M5S 1A8.

A striking correlation has been reported between the human hallucinogenic potency of a series of 4-substituted derivatives of 1-(2,5,dimethoxyphenyl)-2 aminopropanes (2,5 DMA) and their affinity for serotonin receptors assayed in the rat fundus preparation (1). Since it has been established that there are multiple types of central serotonin receptors (2) we decided to investigate the interaction of these compounds for radiolabelled S_1 and S_2 serotonin receptors in rat frontal cortex homogenates. Competition experiments were performed for S_1 receptors labelled by 1.0 nM ^{3}H-LSD in the presence of 10^{-7}M ketanserin (to preclude binding of ^{3}H-LSD to S_2 receptors) and for S_2 receptors labelled by 0.4 nM ^{3}H-ketanserin (3). The IC_{50}s obtained for several hallucinogenic compounds are shown below.

Drugs	IC_{50}s (nM) S_2	S_1
LSD	3.2	2.0
(±) DOI	22	1600
(±) DOM	240	1210
2,4,5 TMA	2010	>10,000
2,5 DMA	5250	1515
4-OET 2,5 DMA	6070	>10,000

Most compounds were more potent at S_2 serotonin receptors. (±) DOI had an exceptionally high affinity for this site. The detailed properties of these and other hallucinogenic compounds, as well as the properties of the resolved enantiomers will be presented.

(1) Glennon, R.A., Young, R., Benington, F. and Morin, R.D., J. Med. Chem. 25, 1163-1168, 1982.
(2) Peroutka, S.J. and Snyder, S.H., Mol. Pharmacol., 16, 687-714, 1979.
(3) Leysen, J.E., Niemegeers, C.J.E., Van Nueten, J.M. and Laduron, P.M., Mol. Pharmacol., 21, 301-314, 1982.

CARDIOVASCULAR REGULATION: MORPHOLOGICAL ASPECTS

334.1 DIFFERENTIAL DENSITY OF ALPHA ADRENERGIC RECEPTORS IN MYOCARDIAL MEMBRANES OF THE DAHL RAT MODEL OF HYPERTENSION. E. Edwards, J. McCaughran and R. Friedman. Department of Psychiatry and Behavioral Science, SUNY at Stony Brook, NY, 11794

Both lines of the Dahl model of hypertension were used in this study: the salt sensitive or DS line which remains normotensive unless exposed to a high salt diet and the salt resistant or DR line which shows no change in blood pressure irrespective of salt intake. Since cardiac activity is under the control of the sympathetic nervous system, the nature, number and the regulation of adrenergic receptors have both pharmacological and therapeutic significance.

We approached the characterization of myocardial alpha adrenergic receptors by two methods: first, by assessing the potencies of various alpha adrenergic ligands (prazosin, phentolamine, yohimbine, clonidine, (-)-norepinephrine) in competing for the binding of ^{3}H-dihydroergocryptine, ^{3}H-DHE (competion curves run at 2.0 nM ^{3}H-DHE); and second by analyzing binding parameters (saturation curves) for three tritiated compounds: the α_1 selective ligand ^{3}H-Prazosin, the $\alpha_2$selective antagonist ^{3}H-Rauwolscine and the non subtype selective ligand ^{3}H-DHE. For the assessment of saturation curves we incubated varying concentrations of ^{3}H-Prazosin (0.06-2.30 nM), ^{3}H-Rauwolscine (0.25-15.0 nM) or ^{3}H-DHE (0.30-7.70 nM) in the presence or absence of 10uM unlabeled Phentolamine.

DR and DS rats were kept on a high salt diet (8% NaCl by weight) for a period of five weeks. High salt intake resulted in significant elevation in blood pressure in DS rats but not in DR rats (DS rats: 166 mm Hg vs DR rats: 116.8 mm Hg). The alpha adrenergic receptors of the Dahl rat myocardium, identified by radioligand binding techniques are mainly of the α-1 receptor subtype. We observed alpha adrenergic ligands to compete with ^{3}H-DHE for binding to cardiac receptors with the order of potency expected for α- receptors, namely: Prazosin > Phentolamine > Yohimbine > Clonidine > (-)-Norepinephrine.

^{3}H-DHE and ^{3}H-Prazosin labeled a single population of sites. The affinity constants or Kd (2.0 nM for ^{3}H-DHE and 0.29 nM for ^{3}H-Prazosin) did not differ between lines but the density of α-1 receptors varied significantly for DR and DS rats (64.6 Fmole/mg protein and 34.5 Fmole/mg protein, respectively). The α-2 selective ligand ^{3}H-Rauwolscine at concentrations up to 15 nM demonstrated negligible specific binding.

Recently, DS rats have been reported to show a deficit of cholinergic activity in myocardial membranes, reflecting a decrease in para-sympathetic input (J.McCaughran et al, submitted to Eur.J.Pharm., 1983). Combined with our present data, the genesis of hypertension in DS rats may be facilitated by a loss of both sympathetic and para-sympathetic inputs.

334.2 DISTRIBUTION OF RENAL AND SPLENIC SYMPATHETIC POSTGANGLIONIC CELL BODIES IN THE CAT. R.L. Meckler and L.C. Weaver. Dept. of Physiol., Mich. St. Univ., E. Lansing, MI 48824.

Previous work in this laboratory has demonstrated that 1) renal and splenic nerves can respond differentially to stimulation of visceral afferent nerves, and 2) basal rates of discharge of renal and splenic nerves are not equally dependent upon supraspinal influences. Different locations of cell bodies of renal and splenic postganglionic nerves within the same ganglion or different distributions among ganglia may determine the specificity of preganglionic innervation of the postganglionic nerves. In this study retrograde axonal transport of horseradish peroxidase (HRP) was utilized to identify renal or splenic postganglionic cell bodies within paravertebral and abdominal prevertebral sympathetic ganglia of 13 cats. Nerves were dissected from the renal or splenic pedicle, cut, and 15-30 mg HRP (Sigma VI) was applied to the moistened central ends for 1 hr. Animals were allowed to recover and survive for 24-48 hr. Sympathetic ganglia were cut into 50 µm sections, reacted with tetramethylbenzidine, and counterstained. Left celiac (L.Cel.), right celiac (R.Cel.), and superior mesenteric (Sup.Mes.) ganglia often were fused into a solar plexus (Sol.Plex.). Sol.Plex. was sectioned as a unit in 4 cats. Sham experiments also were performed in which identical protocols were used, with the exception that nerves were transected 1-2 cm central to the site of HRP application. In cats in which renal nerves were exposed to peroxidase, fewer than 12 neurons per ganglion were labeled from thoracic (T) segments 1-10. Renal neurons were distributed throughout the remainder of the paravertebral chain, with greatest occurrence in lumbar (L) segments (See Table). Renal somata also were located within the Sol. Plex., often closely packed in groups. Following splenic nerve applications of HRP, no more than 5 neurons per ganglion were labeled in ganglia from segments T1-T11. Few splenic neurons were located in the paravertebral chain ganglia; most were distributed uniformly throughout the solar plexus. In summary, cell bodies of renal and splenic nerves are distributed differentially in sympathetic ganglia. Supported by grant HL 21436.

#s of Cells/Ganglion (Range)	Renal		Splenic	
T10	0-11	(6)	0	(4)
T11	0-101	(5)	0-5	(4)
T12	0-232	(4)	0-44	(4)
T13	0-101	(4)	0-28	(4)
L1	2-499	(6)	0-4	(2)
L2	2-455	(6)	0-2	(2)
L3	8-120	(5)	0-1	(2)
Sol.Plex.	11-428	(3)	849-1589	(4)
L.Cel.+	0-440	(6)	3-1841	(4)
R.Cel.+	10	(1)	907	(1)
Sup.Mes.+	0-893	(3)	22-922	(2)

(n): number of cats; +L.Cel., R. Cel., and Sup.Mes., ganglia when sectioned individually.

334.3 LOCATION OF CARDIAC VAGAL PREGANGLIONIC CELL BODIES IN THE FROG. B. J. Pardini* and R. D. Wurster. Dept. of Physiology, Loyola University Stritch School of Medicine, Maywood, IL 60153

The frog parasympathetic ganglion is an often studied model for analysis of pre- and post-ganglionic interactions. However, the central origins of the preganglionic neurons have not been well described. Therefore, the present experiments investigated the location of cardiac vagal preganglionic cell bodies in the frog using the retrogradely transported tracer, horseradish peroxidase.

Frogs were anesthetized by submersion in ice for 60 to 90 min. The chest was opened ventrally and the heart was exposed. Up to 50 µl of a 20% solution of horseradish peroxidase (Worthington, HPOFF) was injected subepicardially with a glass micropipette. Injections were made over the surface of the atria and along the major vessels exiting the heart. After closure of the incision frogs were maintained for 48 hrs before transcardial perfusion with 0.9% saline followed by a mixture of paraformaldehyde (1.0%) and glutaraldehyde (1.25%) in 0.1 M phosphate buffer. Serial sections (40 µ) were cut on a freezing microtome (brainstem was cut in cross section; spinal cord was cut in horizontal section), reacted with tetramethyl benzidine (Sigma), and mounted on slides.

Labeled cell bodies in the brainstem were predominantly located in the region of the dorsal motor nucleus of the vagus from approximately 5 mm caudal to 1 mm rostral to the calamus scriptorius (obex). This area in the frog corresponds roughly to the area between the dorsal motor nucleus and the nucleus ambiguus in mammals. No labeled cells were identified in the spinal cord. Bilateral labeling of neurons in the brainstem was observed when unilateral vagotomy was performed at the time of peroxidase injection.

These results indicate in the frog that cardiac parasympathetic preganglionic neurons: 1) originate primarily in the region of the dorsal motor nucleus, 2) contain crossed components in the brainstem, and 3) do not have a locus of spinal origin. (Supported by NIH Grant HL 27612)

334.4 BRAIN STEM DISTRIBUTION OF THE CAROTID SINUS NERVE IN THE DOG. C.L. Chernicky, K.L. Barnes, C.M. Ferrario and J.P. Conomy. Research Division, Cleveland Clinic Foundation, Cleveland, Ohio 44106

The distribution of the carotid sinus nerve (CSN) afferents was examined in mongrel dogs with horseradish peroxidase (HRP) histochemistry. The nerve bundle containing CSN afferents was identified by the correlation of its discharges with the rising phase of arterial pressure. Multiple microinjections of HRP were made into the intact bundle. [10-25 µl total of 30% HRP (Sigma, Type VI) in TRIS buffer (pH 8.6)]. After 2-4 days the dogs were reanesthetized (pentobarbital, 40 mg/kg, i.v.) and perfused via the ascending aorta with isotonic saline followed by 4.0 l of Karnovsky's fixative and a final wash of 2.5 l of cold sucrose buffer. The brain stem was removed, stored overnight in sucrose buffer at 4°C, and serially sectioned at 50 µm from C_2 to the level of the facial nerve. Sections were processed for HRP histochemistry using tetramethylbenzidine as the chromagen and examined for HRP reaction product with both bright- and darkfield illumination.

In the dog labeled carotid sinus nerve axons enter the lateral brain stem intermingled with unlabeled glossopharyngeal fibers just dorsal to the spinal trigeminal tract and ventral to the restiform body approximately 8 mm rostral to the obex. The HRP positive fibers travel medially until they lie just ventral to the inferior vestibular nucleus where they turn caudally and join the solitary tract (TS). The labeled fibers are present in the ipsilateral TS throughout its extent. At 5 mm rostral to the obex CSN fibers exit from the TS and distribute into the adjacent dorsolateral, dorsomedial and medial regions of the nucleus of the solitary tract (nTS). This pattern of CSN projection in nTS continues to levels caudal to the obex on the ipsilateral side. A few scattered fibers are seen in the area postrema (AP). At levels posterior to the obex HRP reaction product was seen in fibers crossing in the nucleus commissuralis of Cajal and continuing anteriorly into the contralateral nTS and AP. Although the distribution of the canine CSN is similar to that of the cat, the anterior-posterior extent of the pathway is greater in the dog, beginning at 8 mm rostral and ending at 3 mm caudal to the obex. (Supported by NHLBI grants HL-6835, HL-24100 and the Reinberger Foundation).

334.5 THE LOCALIZATION OF GLUTAMIC ACID DECARBOXYLASE (GAD) IMMUNOREACTIVITY IN THE NUCLEUS OF THE SOLITARY TRACT. B. Maley, R. E. Elde, W. H. Oertel*, and D. E. Schmechel* (SPON: George E. Goode). Dept. of Anatomy, Univ. Kentucky Med. Ctr., Lexington, KY 40536; Dept. of Anatomy, Univ. of Minnesota Sch. Med., Minneapolis, MN 55455; Dept. of Neurology, Technical Univ., Munich, FRG; Div. of Neurology, Duke Univ., Durham, NC 27710.

It is generally believed that GAD, a synthesizing enzyme for GABA, is a specific marker for the neurotransmitter, GABA. In a series of eight cats sacrificed by vascular perfusion with 4% paraformaldehyde-0.1% glutaraldehyde in 0.1 M Sorenson's phosphate buffer, pH 7.2 GAD immunoreactivity was visualized as numerous immunoreactive varicosities in various subdivisions of the feline nucleus of the solitary tract (NTS) using the peroxidase, antiperoxidase (PAP) technique. Within the NTS GAD immunoreactivity was differentially distributed in its various subdivisions. The lateral, medial, and commissural subdidvsions and the dorsolateral band of the parvocellular subdivisions contained heavy accumulations of GAD immunoreactivity while the ventrolateral subdivision and the remainder of the parvocellular subdivision possessed lower amounts. At the ultrastructural level GAD immunoreactivity was found within unmyelinated axons, nonsynaptic varicosities, and presynaptic terminals of the NTS. The presynaptic terminals measured 0.5-1.5 um in diameter and were presynaptic to spines, dendrites, and cell bodies of the NTS. The GAD immunoreactivity was associated with clear and granular synaptic vesicles and other membrane bound structures such as mitochondria and microtubules. To investigate whether GAD immunoreactivity was present within neurons of the NTS, several cats were treated with colchicine (500 ug; intraventricular) 48 hours prior to sacrifice. Results of this portion of the study will be discussed.

Results of the present study demonstrate the differential distribution of GAD immmunoreactivity contained within numerous varicosities in the feline NTS. The ultrastructural equivalent of these immunoreactive structures are small axons, nonsynaptic varicosities, and presynaptic terminals contacting NTS neurons and their dendritic trees. The presence of GAD immunoreactive structures in the NTS, an autonomic neural center, suggests a role for it in the central regulation of autonomic functions.

Supported in part by NIH BRSG RR05374 to B. M.

334.6 RELATIONSHIP BETWEEN DORSAL AND VENTRAL MEDULLARY REGIONS WHICH SLOW HEART RATE. S. L. Stuesse, S. E. Fish and K. S. Powell*. Neurobiology Program, N.E. Ohio College of Medicine, Rootstown, Ohio 44272

Parasympathetic pathways in the brain which mediate cardiac inhibition have been well characterized. We previously demonstrated that in the rat, the majority of parasympathetic fibers projecting to the myocardium originate in the nucleus ambiguus (NA) in the ventrolateral medulla. Electrical stimulation (>50Hz, <20µa) slowed heart rate markedly. In the present study we have located a second cardioinhibitory region in rat medulla. We describe this area and present morphological evidence that a portion of it overlaps with areas which project to the cardioinhibitory region of the NA. Rats were anesthetized and placed in a stereotaxic apparatus. The cardioinhibitory portion of the NA was located by electrically stimulating through a horseradish peroxidase (HRP)-filled pipette. When heart rate was slowed, small quantities of HRP were iontophoretically deposited into the NA. Two days later, the rats were reanesthetized and the surface of the dorsal medulla was explored with a steel stimulating microelectrode. An area in the dorsal medulla which slowed heart rate when electrically activated (<20Hz, <20µa) was identified. The most caudal and rostral extent of this area was marked with iron deposits by passing anodal current through the electrode. The rats were processed for HRP using the tetramethylbenzidine method.

The HRP injection identified a major projection to the rostral NA from scattered cells in the medial nucleus of the tractus solitarius (MNTS). These cells extended from the level of the obex to approximately 600µm rostral to the obex. Iron deposits indicated that the dorsal medullary cardioinhibitory region extended from the commisural nucleus of the Cajal (500-1000µm caudal to the obex) into the MNTS (about 300µm rostral to the obex). Thus most of the cells which project to the rostral NA are located in a cardioinhibitory area of the MNTS. However, the cardioinhibitory area of the dorsal medulla extends caudal to the HRP labelled cell bodies. Whether this is due to activation of processes of the more rostral MNTS cells or to activation of interneurons which may be involved in slowing heart rate is not yet known.

Supported by NIH Grant HL23964.

334.7 DISTRIBUTION OF ^{3}H-GABA APPLIED TOPICALLY TO THE VENTRAL SURFACE OF THE MEDULLA OBLONGATA IN THE RAT. J.R. Keeler, C.W. Shults*, T.N. Chase and C.J. Helke. Dept. of Pharmacol., Uniformed Ser. Univ. of the Health Sci., and NINCDS, Bethesda, MD. 20205.

The ventral surface of the medulla oblongata (VSMO) in several mammalian species is selectively sensitive to a variety of agents which alter cardiovascular and ventilatory function. We previously showed that application of GABA to the VSMO in artificially ventilated rats produced hypotension and bradycardia which were mediated primarily by the sympathetic nervous system. The area of the VSMO most sensitive to GABA was a region just lateral to the pyramids and between the trapezoid body and the exits of the hypoglossal nerves. This region is analogous to "Schlaefke's area" in the cat. It is not known however, if the cardiovascular responses were produced by GABA acting at the area of drug application or the result of transport or diffusion of GABA to other structures more noted for their roles in cardiovascular regulation. We sought to localize the spread of topically applied GABA by in vivo autoradiography.

The VSMO was surgically exposed in anesthetized, artificially ventilated S/D rats. Filter paper pledgets (1 X 1.5mm) were soaked with a solution of ^{3}H-GABA, cold GABA, and artificial CSF (0.39 µmol, 0.1µCi/pledget). Bilateral pledget application to the VSMO area of greatest GABA sensitivity resulted in the characteristic hypotension and bradycardia. When maximal responses were obtained (2 min), blood samples were drawn, the brains immediately removed and frozen. Slide-mounted coronal sections (20 µm) were exposed to LKB Ultrofilm ^{3}H for 4 weeks, and adjacent sections were stained with thionin. Scintillation counting showed absence of radioactivity in peripheral plasma. Autoradiograms showed approximately 1 mm penetration of ^{3}H into the parenchyma and 0.75 mm superficial rostro-caudal and lateral spread. Histological examination showed that the heaviest labelling was at the site of application, and was primarily localized to the ventral half of the lateral paragigantocellular nuclei (Anat. Embryol. 161:355, 1981). Additional labelling was seen at the ventrolateral aspects of the pyramids and principal olives, and at the ventromedial aspects of the tracts of the spinal trigeminal nuclei and caudal facial nuclei. The superior parolivary nuclei and the ventromedial aspects of the rostral lateral reticular nuclei were very lightly labelled. No labelling was seen rostral or caudal to these nuclei, respectively.

These results suggest that the site of action of the cardiovascular effects of GABA topically applied to the VSMO is at or near the surface of "Schlaefke's area". (Supported by NIH grant NS-19317).

334.8 CEREBELLAR AND MEDULLARY AFFERENTS TO THE PARAMEDIAN RETICULAR NUCLEUS: AN HRP STUDY IN THE CAT, K. Elisevich*, A. W. Hrycyshyn* and B. A. Flumerfelt. Department of Anatomy, The University of Western Ontario, London, Canada N6A 5C1.

The paramedian reticular nucleus (PRN) has been shown in cats to be functionally associated with both the fastigial nucleus (fastigial pressor response) and the carotid sinus nerve (baroreceptor reflex) as well as the vestibular nuclear complex (static and dynamic postural responses). The neuroanatomical organization which underlies these functional associations was studied using the retrograde horseradish peroxidase (HRP) method. The PRN was injected transependymally with wheat germ agglutinin-conjugated HRP using the capillary-diffusion technique. After survival periods of 72 - 96 hours, the sites of afferent projection were identified using the tetramethylbenzidine method.

The deep cerebellar nuclei project in a predominantly contralateral fashion onto the PRN with both the fastigial and dentate nuclei providing the majority of fibers. Labeled cells were found throughout the anteroposterior extent of the dentate nucleus whereas a large number of the fastigial fibers originated posteriorly in the dorsal aspect of the fastigial nucleus. A smaller ipsilateral fastigial afferent component was also present. Nuclear groups within the solitary complex containing labeled cells were found bilaterally with a contralateral predominance. Most of the label was found within the ventrolateral and commissural nuclei, both of which have previously been found to be sites of termination of carotid sinus fibers. Both lateral vestibular nuclei contributed the majority of fibers from the vestibular complex with a slight ipsilateral preponderance. A sparser presentation of label was found in the medial and inferior vestibular nuclei as well as in cell group 'Y'. Bilateral afferent projections from the nucleus intercalatus (of Staderini) which itself has been shown to receive vestibular afferents were present. A large number of labeled cells within both lateral tegmental fields were situated largely medially with an ipsilateral predominance. Additional sources of afferent fibers included the contralateral PRN and the medullary raphe nuclei.

This data suggests a neuroanatomical substrate by which cardiovascular and postural information may be mediated by the PRN in adjusting systemic arterial pressure according to various postural attitudes.

Supported by the Medical Research Council of Canada.

334.9 AN ANATOMICAL AND NEUROCHEMICAL STUDY OF THE LOCUS COERULEUS IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR) AND WISTAR-KYOTO RAT (WKY). L.R. Rubin*, J.A. Weyhenmeyer, S.Y. Felten, and D.L. Felten. Depts of Anatomy, Ind. Univ. Sch. Med., Indianapolis, IN 46223, Univ. of Illinois Coll. Med., Urbana, IL 61801, and Univ. Rochester Sch. Med., Rochester, NY 14642.

Physiologic evidence points towards the locus coeruleus (LC) as one of several brain stem nuclei where noradrenergic neurotransmission may modulate blood pressure. Although the LC does not directly regulate sympathetic outflow through the intermediolateral cell column of the spinal cord, this noradrenergic nucleus has abundant projections to brain stem, hypothalamic, and limbic nuclei which in turn are major regulators of autonomic outflow. Altered norepinephrine levels have been found in hypothalamic nuclei in the SHR which receive LC projections, in several studies, including the present study. We examined the anatomy of LC neurons in 16 week old SHR and WKY rats with glyoxylic acid histofluorescence for localization of norepinephrine, with fluorescent Nissl stains (ethidium bromide and acridine orange) in frozen sections for cellular size and architecture, and with a Golgi-Cox technique for dendritic and somatic cytoarchitectural characteristics. The levels of norepinephrine, dopamine, and serotonin were measured in LC and several other micropunched brain stem and hypothalamic nuclei with high performance liquid chromatography with electrochemical detection. Angiotensin-II fiber profiles were localized with light microscopic immunocytochemistry.

Anatomically, the most conspicuous difference was the greater dendritic branching and greater total extent of dendritic arborization in the transverse plane in LC neurons of SHR compared with WKY rats, noted in Golgi-Cox specimens. Formation of small dendritic clusters was not found in either strain, despite their presence in normal Wistar rats. Norepinephrine localization and levels were not significantly different in locus coeruleus or subcoeruleus between SHR and WKY rats, nor were levels of dopamine and serotonin in these areas. Immunocytochemically identified fiber profiles of angiotensin-II were more abundant in LC in the SHR than in the WKY rat. These findings suggest that neuronal architecture in LC is different in the SHR compared with the WKY rat, but that these cellular differences are not accompanied by altered levels or localization of the biogenic amines. The increase in angiotensin-II fiber profiles in the SHR suggests that peptide-monoamine interactions may be increased in this important system in the SHR. Both ultrastructural studies and neurochemical turnover studies are needed to further explore these differences, and to explain the alterations in norepinephrine neurotransmission in the hypothalamus of the SHR.

Supported by NIH grant HL27757.

334.10 THE SUBNUCLEAR ORGANIZATION OF THE EFFERENT CONNECTIONS OF THE PARABRACHIAL NUCLEUS IN THE RAT. C.E. Fulwiler* and C.B. Saper, (Spon: C. Hughes), Depts. of Neurology & Anatomy/Neurobiology, Washington University School of Medicine, St. Louis, MO 63110

The efferent projections of the parabrachial nucleus (PB) were studied in detail using the technique of retrograde transport of wheat-germ agglutinin-conjugated horseradish peroxidase. Injections were made into fourteen terminal fields in a series of seventy rats and the brains were processed by the TMB method of de Olmos et al. The patterns of retrograde labeling correspond in large part to subdivisions of PB which can be distinguished on cytoarchitectural grounds. The lateral division of PB consists of five distinct groups of neurons. These groups provide the major output to the basal forebrain. Some of these groups have limited projections, for example, internal lateral to the intralaminar thalamic nuclei; extreme lateral primarily to the bed nucleus of the stria terminalis; and supralateral to the hypothalamus. The largest projection from central lateral is to the median preoptic nucleus, but some neurons also project to septum, hypothalamus, and paraventricular thalamus. External lateral projects to zona incerta, amygdala and nucleus basalis. In contrast to the lateral division, the medial division of PB consists of a heterogeneous population of neurons which primarily innervate insular cortex and associated areas. One exception is a relatively homogenous, narrow band of large, multipolar neurons called external medial PB which projects bilaterally to the ventromedial basal thalamic nucleus. Two specific patterns of labeling include portions of both medial and lateral PB. After injections into the nucleus basalis, labeled neurons in external lateral and medial PB surround the lateral pole of the superior cerebellar peduncle in a confluent group. In the caudalmost portion of PB, a dense group of small neurons encircling the waist of the peduncle were labeled after injections in cortex, amygdala, nucleus basalis and zona incerta. In the Kolliker-Fuse subdivision of PB, neurons projecting to the region of the nucleus of the solitary tract were found primarily rostral to the neurons which projected to spinal cord, though there was some overlap.

Supported by grants from NINCDS NS18669 and NS0631 and from the McKnight Foundation and the American Parkinson Disease Association.

334.11 AFFERENT CONNECTIONS OF THE MEDIAN PREOPTIC NUCLEUS: ANATOMICAL EVIDENCE FOR A CARDIOVASCULAR INTEGRATIVE MECHANISM IN THE ANTEROVENTRAL THIRD VENTRICLE (AV3V) AREA. D. Levisohn* and C. B. Saper. Department of Neurology, Washington University School of Medicine, St. Louis, Missouri 63110

A rapidly accumulating body of evidence indicates that the AV3V area plays a critical role in normal regulation of fluid volume and in the development of experimental hypertension, but little is known of the neural mechanisms responsible for these observations. We have injected the tissue surrounding the AV3V with small amounts (3-20nl) of a 1% solution of wheat germ agglutinin-horseradish peroxidase conjugate in order to define the afferent connections of cell groups in this region. Following 48 hours survival, tissue was processed by the TMB method of De Olmos, et al.

Within the AV3V area, the median preoptic nucleus (MnPO) had a unique set of inputs from areas implicated in cardiovascular control. (i) Many neurons were retrogradely labeled in the subfornical organ, particularly in its anterior portion, and in the rim of its posterior part. (ii) Retrogradely labeled neurons were seen in the parvocellular (autonomic) portions of the paraventricular nucleus of the hypothalamus. (iii) A dense cluster of retrogradely labeled neurons was seen in the lateral parabrachial nucleus. (iv) Retrogradely labeled neurons were seen in the nucleus of the solitary tract primarily in the area just medial to the solitary tract, near the level of the obex. This area is similar to the region which receives baroreceptor afferents in the cat. (v) Scattered labeled neurons were seen in the ventrolateral medulla, at the same rostrocaudal level as the nucleus of the solitary tract labeling. Control injections into other AV3V structures surrounding MnPO demonstrated that none of these inputs was labeled due to uptake by fibers of passage, or involvement by the injection of adjacent areas.

The median preoptic nucleus is the only AV3V structure which receives so wide-ranging a set of inputs from areas involved in cardiovascular control. On this basis, it seems likely that many of the cardiovascular perturbations produced by AV3V lesions are due to injury to MnPO or to its connections.

Supported by USPHS grants NS0631 and NS18669, and by grants from the American Parkinson Disease Association and the McKnight Foundation.

RESPIRATORY REGULATION

335.1 HYPOGLOSSAL AND RECURRENT LARYNGEAL RESPONSES TO PULMONARY STRETCH RECEPTOR AND SUPERIOR LARYNGEAL INPUTS. A.L. Sica, D.F. Donnelly*, H. Zhang*, and M.I. Cohen. Dept. Physiol., Albert Einstein Col. Med., Bronx, N.Y. 10461.

The effects of pulmonary stretch receptor (PSR) and superior laryngeal (SL) afferents on the efferent discharges of the hypoglossal (HYP) and recurrent laryngeal (RL) nerves were studied with both whole nerve and single fiber recordings. Cats were decerebrated, paralyzed, and artificially ventilated with a cycle triggered pump. The discharges of the HYP and RL differed from that of the phrenic (PHR) in several ways: a) they had earlier onsets, 40-120 msec before PHR; b) discharge patterns of the HYP and RL were decrementing and plateau-like, respectively; c) expiratory activity was usually observed in the RL discharge. When inflations were withheld, resulting in elimination of phasic PSR input, there was an increase in HYP and RL activity, and both discharge patterns became augmenting. Four discharge patterns were observed for RL fibers: a) phasic inspiratory, b) tonic inspiratory, c) early expiratory, d) expiratory-inspiratory. Withholding inflation increased the discharges of all types; RL phasic inspiratory fibers changed from a plateau-like pattern to an augmenting pattern. HYP fibers were all phasic inspiratory with decrementing patterns which became augmenting upon withholding inflation. SL stimulation (.05 msec shock, 40-120 ua) at various times during inspiration elicited an excitatory response followed by a suppression of activity in the ipsilateral whole HYP and RL, and in the contralateral PHR. The mean latencies for excitation were: 4.2, 5.6, 7.9 msec for the PHR, HYP, RL, respectively; suppression periods were similar for all nerves, about 30 msec. Phasic inspiratory fiber discharges of both the HYP and RL were usually suppressed (13-37 msec) by SL stimulation; in only a few cases was suppression preceded by excitation. Our results indicate: a) HYP and RL discharges are inhibited in a graded manner by PSR inputs, with the HYP more strongly inhibited; b) SL afferents exert similar effects on the neural populations driving the PHR, HYP, and RL, but with different latencies. These effects suggest that both PSR and SL afferents affect upper airway patency by changing HYP and RL discharges. (Supported by USPHS Grants HL-27300 and HL-07060.)

335.2 INHIBITION OF MEDULLARY INSPIRATORY DRIVE BY INTERCOSTAL MUSCLE TENDON ORGANS. D.C. Bolser*, B.G. Lindsey and R. Shannon* (SPON: M. Nolan). Dept. Physiol., Col. Med., Univ. S. Fl. Tampa, 33612

Electrical stimulation of intercostal nerve Group I afferents has an inhibitory effect (reduced activity) on medullary dorsal and ventral respiratory group inspiratory neurons, resulting in a decreased motor drive to the inspiratory muscles. Whether the afferent fibers arise from primary muscle spindle endings (Ia fibers) and/or tendon organs (Ib fibers) is not known.

A technique was developed which allowed stimulation of tendon organs (TO) in intercostal muscles (IM) without stimulation of the muscle spindles. Thus, experiments were conducted to determine if TO in external and internal IM have an inhibitory effect on medullary inspiratory drive. Inspiratory activity was monitored from a C_5 phrenic rootlet. Experiments were performed on anesthetized (Dial) vagotomized, ventilated cats.

An isometric muscle contraction was elicited in a single intercostal space (T6) by electrical stimulation (3 pulses, 0.1ms pulse duration, 0.7-6.8V) of the peripheral cut end of the T6 ventral root (VRS). The two ribs of the space were clamped after surgically separating them from adjoining IM. The muscle twitches resulted in a transient reduction or premature termination of phrenic activity at latencies of 24-35 ms. This phrenic nerve response was still present with only the external or internal IM innervated, but was eliminated when both the external and internal IM were denervated.

The response of muscle spindle endings (MSE) during the VRS elicited muscle contraction was determined by monitoring afferent fibers in split dorsal roots. MSE (59 in 13 cats) were identified by their ability to follow low amplitude (80-220um), high frequency (100 Hz) vibration of the IM. All MSE decreased their firing rate during the muscle contraction. Other afferent fibers (23 in 13 cats) were recruited or increased their firing rate during muscle contraction, and most likely were from TO; their activity increased during the rising phase of the muscle twitch. Other receptors (i.e., pacinian corpuscles, free nerve endings) in the muscle may also have been stimulated, but our previous intercostal nerve studies strongly suggested that these receptors do not have an inhibitory effect on inspiratory activity. Considered together, these data provide evidence that TO are responsible for the reduction in inspiratory activity during the VRS elicited muscle contraction.

Since we know from our previous studies that intercostal muscle Group I afferents reduce phrenic activity via brainstem and not segmental pathways, we conclude that external and internal intercostal muscle tendon organ afferent information ascends to the brainstem and has an inhibitory effect on medullary inspiratory drive. (Supported by NIH Grant HL-17715)

335.3 EXCITATORY EFFECTS OF GLUTAMATE, SUBSTANCE P AND ERP ON SINGLE NEURONES INVOLVED IN RESPIRATORY CONTROL IN THE NUCLEI OF THE TRACTUS SOLITARIUS. J.L. Henry and B.J. Sessle (Spon: R.B. Malmo), Department of Physiology, McGill University, Montréal, Québec, H3G 1Y6 and Faculty of Dentistry, University of Toronto, Toronto, Ontario, M5G 1G6.

Glutamate and substance P have both been implicated in central mechanisms of cardio-respiratory control, and they are found in high concentration in the nuclei of the tractus solitarius (NTS). We therefore undertook an electrophysiological study to determine their effects on respiration-related neurones in the NTS of paralyzed, chloralose-anaesthetized cats. Extracellular single unit spikes were recorded from one barrel of multibarrelled micropipettes. Barrels for iontophoresis contained Na-L-glutamate (1M, pH 7 4) and substance P or eledoisin-related peptide (ERP, a partial homologue of sP). Peptides were dissolved as 1 mM solutions in 165 mM NaCl at pH 5.5. All neurones were functionally identified (Sessle et al., Brain Res. 216: 146, 1981) as either respiratory (n = 42; fired rhythmically in phase with phrenic nerve activity) or as interneurones implicated in respiratory tract reflexes (n = 16; no rhythmic activity, but responded orthodromically to electrical stimulation of vagal and/or superior laryngeal nerves). Glutamate responses were typical of the fast excitation described elsewhere in the CNS However, glutamate showed a differential effect on respiratory neurones. Whereas all 16 reflex interneurones tested were excited by < 30 nA, the respiratory neurones were of "sensitive" (< 30 nA, n = 22) and "insensitive" (> 60 nA or unresponsive, n = 18) types. Substance P and ERP shared similar effects: they excited 12 of 19 respiratory neurones tested and all of the 6 reflex interneurones tested. The excitation began within 15-30 sec of the beginning of current ejection, increased in magnitude throughout the period of ejection and gradually disappeared over the 30-120 sec after ejection was stopped. These findings that glutamate and substance P have excitatory effects on respiratory neurones and reflex interneurones support the view that they may be chemical mediators of synaptic transmission in the nuclei of the solitary tract, in pathways related to respiratory control.

(Supported by the Canadian Medical Research Council)

335.4 SHORT TIME SCALE CORRELATIONS BETWEEN PONTINE AND MEDULLARY RESPIRATORY NEURONS OF THE CAT. L.S. Segers*, R. Shannon* and B.G. Lindsey. University of South Florida, Department of Physiology, Tampa, Florida 33612.

Interactions between pontine and medullary respiratory neurons have been inferred from anatomical, lesioning, stimulation and single unit antidromic studies. We have used cross-correlation analysis of simultaneously recorded pontine (n. parabrachialis medialis, Kölliker-Fuse n.) and ipsilateral medullary (n. ambiguus, n. retroambigualis, retrofacial n.) respiratory neurons to detect and evaluate functional interactions between concurrently monitored pontine and medullary respiratory neurons. Phrenic nerve activity and brainstem respiratory neuron activity were recorded extracellularly in decerebrate, vagotomized, paralyzed and artificially ventilated cats. Two hundred and sixty pairs, each pair consisting of a pontine and a medullary respiratory neuron, have been analyzed; the results are summarized below.

CENTRAL PEAK		PEAK TO ONE SIDE OF ZERO		TROUGH TO ONE SIDE OF ZERO		HIGH FREQUENCY OSCILLATION		CORRELATION TOTALS	
N	%	N	%	N	%	N	%	N	%
3	1.15	5	1.92	1	0.38	7	2.69	16	6.15

The small number of correlated spike trains suggests that monosynaptic connections between the pontine and medullary respiratory neurons in the areas surveyed are "weak" or rare. The presence of a central peak in a cross-correlation histogram provides evidence for either an input from an unobserved source that is shared by the two neurons under analysis or mutually excitatory interactions. The existence of a peak to one side of zero in a cross-correlogram may be due to an axonal projection from one neuron to the other or to a shared input that, due to differences in times of conduction, influences one cell before the other. It is of interest to note that the neurons of six of the eight pontine-medullary pairs which exhibited a central peak or a peak to one side of zero had their maximum rates of activity during different phases of the respiratory cycle. High frequency oscillations were observed primarily between respiratory neurons of the same type. Previous hypotheses regarding interactions between pontine and medullary respiratory neurons have suggested that medullary inspiratory cells excite pontine inspiratory cells and that medullary inspiratory cells are inhibited by pontine early expiratory neurons. Our data raise the possibility that interactions between pontine and medullary respiratory neurons may involve neurons that exhibit diverse patterns of respiratory activity.

335.5 SHORT TIME SCALE CORRELATIONS BETWEEN DISCHARGES OF DORSAL INSPIRATORY (I) NEURONS AND PHRENIC (PHR) MOTONEURONS. M.I. Cohen and J.L. Feldman. Depts. of Physiol. Albert Einstein Col. Med., Bronx, NY 10461, and Northwestern Univ., Chicago IL 60611.

In decerebrate paralyzed cats ventilated with a cycle triggered pump that delivered inflations during the central inspiratory (I) phase, activity of 82 dorsal I neurons was recorded in the region of ventrolateral nucleus of tractus solitarius. The neurons were classified by their responses to withholding inflation during I, which eliminated phasic lung afferent input: a) excitation by inflation [inflation(+)], b) no significant effect of inflation [inflation(0)], and c) depression by inflation [inflation(-)]. The inflation(+) neurons (corresponding to "I-beta" neurons) were further classified, on the basis of the bimodal distribution of discharge onset times, into early-onset or late-onset. Crosscorrelation histograms (CCH) of unit firing vs. whole PHR nerve (contralateral and ipsilateral) activity on a short time scale were computed; the majority of these had high frequency oscillations (HFO) with mean period of 14.6 msec. The CCHs for the different groups of neurons were analyzed with respect to: a) Laterality of strength of correlation. The relative amplitudes of the main correlation peaks in the ipsilateral vs. contralateral CCHs were compared; by a subtraction procedure which cancelled the HFO, peaks superimposed on the HFO were detected. b) Lag of the main correlation peak. The neurons fell into two broad groups: 1) The inflation(0) and inflation(+) neurons (both early- and late-onset) had CCH peaks with lags of 3-4 msec and which were laterally asymmetrical, i.e. the contralateral peak was larger than the ipsilateral peak. The contralateral peak component had a short latency (1.5-2.5 msec) and rapid rise time (ca. 1 msec), suggesting monosynaptic excitation of PHR neurons by the medullary neuron. 2) The inflation(-) neurons (of both augmenting and decrementing types) had CCH peaks with shorter lags (1-2 msec) which were laterally symmetrical. Thus, both inflation(0) neurons ("I-alpha") and inflation(+) neurons ("I-beta, both early- and late-onset) excite (probably monosynaptically) PHR motoneurons, a result which casts doubt on the hypothesis that "I-beta" neurons mediate the "I off-switch" mechanism.

(Supported by USPHS Grants HL-20800 and HL-27300.)

335.6 PROJECTION OF PHRENIC NERVE AFFERENTS TO THE CAT SENSORIMOTOR CORTEX. P.W. Davenport*, F.J. Thompson & A.N. Freed* (Spon. by: R. Reep). Depts. of Physio. Sci. & Neurosciences, Univ. of Fl, Gainesville, FL 32610.

The majority of research to date on the central neural control of respiration has been focused primarily on medullary and pontine nuclei in an effort to define the mechanisms responsible for the generation of respiratory rhythm and the factors that modulate it. Although it has been recognized that higher brain centers affect respiration there is a paucity of specific studies dealing with regions of the brain above the pons. There are no reports on the projection of phrenic nerve afferents to the cerebral cortex. The present study was designed to 1) demonstrate the projection of sensory information to the sensorimotor cortex from the diaphragm via the phrenic nerves and 2) to map the primary projection loci.

Cats were anesthetized with α-chloralose, paralyzed and mechanically ventilated. The animal's body temperature, arterial blood pressure and gases were monitored throughout the experiment. The phrenic nerve (right or left) was isolated in the thorax and the most distal 2cm stimulated by bipolar electrodes. The rostral and parietal cerebral cortex was exposed bilaterally via a craniotomy. A 2x2mm grid pattern was used to map the cerebral hemispheres for cortical evoked potentials (CEP) elicited by stimulation of the whole phrenic, C-5 portion of the phrenic, superficial and deep radial, sural, gastrocnemius and major splanchnic nerves. Each point was investigated by a silverball recording electrode. A CEP map was obtained by repetitive sampling the evoked responses elicited by the stimulation of the various nerves. The cortical position that had the largest primary evoked response was marked and the precise region identified histologically.

Electrical stimulation of the whole phrenic nerve produced primary evoked potentials in areas 4γ, 3a and SI. The primary afferent projection sites were in the areas corresponding to the trunk and forelimb. Electrical stimulation of the C-5 portion of the phrenic nerve resulted in CEP with larger amplitude and more widespread distribution of primary waveforms. The primary cortical loci of the C-5 portion and whole phrenic appeared in convergent cortical regions. The conduction velocities of the afferents activated by stimulation of the whole phrenic at the CEP threshold and recorded at the C-5 portion of the phrenic were 53.7 and 40.1 m/sec.

These experiments demonstrate afferent projections from the phrenic nerve to the sensorimotor cortex. The primary afferent projection loci correspond to related somatotopic projections The authors propose that the projection of diaphragmatic afferents to the cerebral cortex provides the afferent substrate for cortical participation in the control of respiration

335.7 PROJECTIONS FROM THE ROSTRAL PONS TO BULBAR RESPIRATORY NEURONS. J. E. Remmers*, D. W. Richter*, J. P. Baker, R. Takeda* and K. P. Madden*. Depts. of Medicine and Physiology, Univ. of Texas Medical Branch, Galveston, TX 77550.

The expiratory phase of the respiratory cycle is comprised of two components, a post inspiratory (PI) period followed by a neural expiratory period (Richter, D. W., J. Expt. Bio. 100: 93-107, 1982). PI neurons depolarize abruptly at the end of the ramp portion of the phrenic neurogram and repolarize progressively during the PI period. They recieve post-synaptic inhibition during neural expiration and inspiration. We examined the response of these neurons to the expiratory facilitatory input caused by electrically stimulating the rostral pons in the region of the nucleus parabrachialis medialis. Membrane potential (MP) of PI neurons was recorded intracellularly using glass micro-pipettes in pen-tobarbital anesthetized, paralyzed, cats ventilated by a servo-respirator. PI neurons were found at the level of the obex, and none were activatable anti-dromically from the vagus or spinal cord. The occurrence of inhibitory post-synaptic potentials (ipsps) identified by a chloride reversible hyperpolarization associated with a decrease in "input" resistance was consistently observed during neural inspiration and was usually apparent during neural expiration. A single rostral pontine stimulus elicited an action potential in PI neurons only during the post-inspiratory period; the amplitude of the epsp during neural inspiration and expiration was not sufficient to adequately depolarize the cell to its firing threshold. The rate of change of MP during the upstroke of the epsp was greater during the post-inspiratory period than during neural inspiration or expiration. The apparent gating of action potentials in PI neurons probably results from the action of ipsps to hyperpolarize the membrane and increase its conductance during the periods of neural inspiration and neural expiration.

The response of PI neurons to a rostral pontine stimulus resembles its response to a superior laryngeal nerve stimulus (Remmers, J. E. et al., Physiologist 28: 188, 1982). These neurons appear to be a site of convergence for processing inputs that prolong expiration by increasing the duration of the PI period. Our findings are consistent with the hypothesis that PI neurons are reponsible for or associated with "irreversible" off switching of inspiration. (Supported by DHHS 1RO1 HL 27520-01).

335.8 PROJECTIONS FROM THE BRAINSTEM TO THE PHRENIC MOTOR NUCLEUS IN THE CAT. J.R. Holtman, Jr., W.P. Norman and R.A. Gillis. Departments of Pharmacology and Anatomy, Georgetown University Schools of Medicine and Dentistry. Washington, DC 20007.

The phrenic motor nucleus (PMN) represents the final motor outflow of the respiratory system responsible for the rhythmic contraction of the diaphragm. The activity of phrenic motor neurons is controlled by input from the brain. The purpose of our study was to identify the central nuclei that project to the PMN by employing the technique of retrograde tracing with a fluorescent dye.

Cats (2-4 kg) of either sex were anesthetized with ketamine (35 mg/kg) and a laminectomy was performed to expose the fourth, fifth and sixth segments of the cervical spinal cord (C4-6). The animals were paralyzed (pancuronium, 200 ug/kg) and artificially respired through a tracheal cannula during the time of fluorescent dye injection. Propidium Iodide (3%) was delivered by microiontophoresis (2 uA, 5-10 min) into the C4-6 segments. Specifically, injections were made 0.9 mm lateral to the posterior median sulcus and 3.5 mm ventral to the dorsal surface of the spinal cord. The animals were allowed to recover for four days to allow retrograde transport of the tracer. At this time, the cats were deeply anesthetized with pentobarbital (50 mg/kg) and perfused through the ascending aorta with 4% paraformaldehyde in 0.1 M phosphate buffer (pH=7.4). The brain and spinal cord were removed and placed in 5% sucrose in 0.1 M phosphate buffer for 24 hrs. The tissue was frozen and cut (14 um sections) on a cryostat. The tissue sections were then viewed under the flourescence microscope at an excitation wavelength of 550 nm and the fluorescent cell bodies were photographed.

Cell bodies containing Propidium Iodide were found mainly within the brainstem. Within the medulla, fluorescent neurons were identified in the raphe pallidus (B1) and obscurus (B2). Labeled neurons were also found contralaterally in the ventrolateral nucleus of the solitary tract and in the nucleus ambiguus/retroambiqualis complex. Within the pons, fluorescent neurons were identified in the raphe magnus (B3) and ipsilaterally in the Parabrachial and Kolliker-Fuse nuclei. Finally, some fluorescent neurons were identified in the raphe dorsalis (B7).

The results of this study are in agreement with electrophysiological studies (Cohen, Physiol. Rev. 59: 1105, 1979) indicating that the ventrolateral nucleus of the solitary tract, the nucleus ambiguus/retroambiqualis and the Parabrachial and Kolliker-Fuse nuclei project directly to the PMN. In addition, these findings suggest a possible role for brainstem raphe nuclei in respiratory function. (HL 29562).

335.9 EFFECT OF ELECTRICAL STIMULATION OF THE RAPHE NUCLEI ON RESPIRATION IN THE CAT. Y.M Hernandez*, J.R. Holtman, Jr., W.P. Norman R.A Gillis and K.L. Dretchen. Departments of Pharmacology and Anatomy, Georgetown University Schools of Medicine and Dentistry, Washington, DC 20007.

The effects of electrical stimulation of the raphe nuclei on respiration have been studied in the spontaneously breathing cat. Animals (1.75-3.5 kg) of either sex were anesthetized with alpha-chloralose (80 mg/kg) and tracheal airflow was measured with a Fleisch No. 1 pneumotachograph. The flow signal was integrated to obtain tidal volume. Respiratory rate could then be determined from the tidal volume tracing. In addition, a femoral artery was cannulated to monitor blood pressure. Arterial pCO_2, pO_2, pH, and HCO_3^- were determined prior to electrical stimulation.

Stimulation of the raphe pallidus (n=5) using currents ranging from 5 to 110 uA delivered at 50 Hz, 1 msec, for 60 sec resulted in marked changes in respiratory rate. Initial increases in rate were observed at low currents (9 to 36 uA). Stimulation currents ranging from 13.5 - 45 uA resulted in maximal increases in respiratory rate from 17 + 2 to 22 + 2 breaths/min over the 60 sec stimulation period. In addition, a decrease in arterial blood pressure was recorded in 3 out of 5 cats. In contrast to the effects seen with the raphe pallidus, stimulation of the raphe magnus (n=4) using currents ranging from 18-250 uA at 50 Hz, 1 msec, for 30 sec resulted in greater changes in tidal volume than in respiratory rate. A decrease in tidal volume was observed initially at currents between 36-54 uA. Stimulation currents ranging from 72 to 162 uA resulted in a maximal decrease in tidal volume from 31 + 1 to 12 + 1 ml during the first 5 sec of electrical stimulation. Complete respiratory inhibition was produced at currents ranging between 90-180 uA. This inhibition could be maintained for the entire 30 sec period by further increasing the intensity of the stimulation. No consistent blood pressure responses were observed during stimulation of the raphe magnus.

The results of these studies correlate well with the changes we have observed in phrenic nerve activity during electrical stimulation of the raphe nuclei in the artificially ventilated cat (Dretchen et al. Fed. Proc. 42, 331, 1983). These data suggest that the raphe pallidus and magnus may be important central nuclei involved in respiratory function. (Supported by HL 29562.)

335.10 POWER SPECTRAL ANALYSIS OF EFFERENT PHRENIC ACTIVITY IN NEONATAL SWINE, P.M Gootman, H.L. Cohen, A.M. Steele*, A.P. Rudell*, L.P. Eberle*, Dept. Physiol. Downstate Med. Cent. SUNY, Bklyn, N.Y. 11203.

Efferent phrenic nerve (PHR) activity (monitor of central respiratory oscillator) was monophasically recorded in piglets < 1 day-39 days of age, anesthetized with Althesin (4 mg/kg/hr), immobilized with C-10 and artificially ventilated. Recordings were made on magnetic tape of left and/or right PHR activity (bandpass 10 or 30 Hz to 10,000 Hz), integrated PHR activity (time constant 0.1 sec), intratracheal pressure (ITP), blood pressure, EKG, and markers indicating phases of artificial ventilatory cycle. Computer analyses (PDP 11/45) included correlation and averaging techniques, as well as power spectral analysis. Sampling was triggered during inspiratory (I: onset of PHR burst) or expiratory (E: offset of PHR discharge phase. Power spectral densities of PHR activity were investigated at sampling intervals of 0.1 to 4.0 msec and 128-2048 points using the Fast Fourier Transform* (FFT). Examination of initial power spectra indicated that the predominant activity fell within a frequency band of 10-400 Hz. Two 1024 data point FFTs were computed per epoch, with a sampling rate of .244 msec. The Nyquist foldover rate determined the low pass filter setting of 2kHz. Averages of 50-200 epochs were plotted during I or E. Hanning windows* were applied to reduce side lobe leakage. Results were plotted as normalized average power (μV^2) per frequency interval (Hz). During I, peaks of power at higher frequencies (ca. 150 Hz) were observed in older piglets when compared to younger animals, i.e., < 1 day old animals had greatest power at ca. 100 Hz. During E, no peaks of power were observed at any frequency for all ages studied. Examination of PHR activity during hyperoxia, hypoxia, normoxia and hypercapnia revealed maturational differences in responses of the central respiratory oscillator to these stresses. Hypoxia and hypercapnia increased power over a limited range of frequencies which, at present, appears to be age-related. In contrast, hyperoxia decreased power at all ages. The results, to date, indicate that changes in the power of the frequency spectrum can be used to demonstrate postnatal maturation of the central respiratory oscillator. (Supported by NIH Grant #HL-20864).

*Brigham, E.O., "The Fast Fourier Transform", Prentice-Hall, 1974

335.11 CHARACTERISTICS OF RESPIRATORY NEURONS IN THE GUINEA PIG. G.B. Richerson* and P.A. Getting (SPON: L. Donaldson). Dept. of Physiol. Biophys., Univ. of Iowa, Iowa City, IA 52242.

Neural mechanisms for the generation of the mammalian respiratory rhythm have been studied extensively using extracellular techniques in situ. The guinea pig offers a demonstrated usefulness for in vitro studies of respiratory areas in the brainstem (see Dekin and Getting, Neurosci. Abtsr.,1983), however, little is currently known about the distribution and characteristics of respiratory neurons in this species. Using extracellular recording techniques, we have examined the organization of medullary respiratory centers of the guinea pig.

Animals were anesthetized using methoxyflurane, while blood pressure and tracheal pressure were continuously measured. Phrenic nerve activity was monitored using a cuff electrode implanted in the neck. The medulla was exposed using an occipital craniotomy and cerebellar aspiration. The animal was paralyzed (Flaxedil), given a pneumothorax, and actively respirated. Single unit activity was recorded using tungsten microelectrodes. Neurons were characterized by their cycle triggered histograms constructed from activity recorded during three experimental conditions: 1) phrenic nerve triggered lung inflation, 2) withholding lung inflation, and 3) active respiration independent of phrenic nerve activity.

Most respiration-related units (RRUs) located in the dorsal respiratory group (DRG) (nucleus tractus solitarius) fired during inspiration. Inspiratory cells with incrementing, decrementing, and constant frequency discharges were observed. Responses to lung inflation included "inflation (0)", and "inflation (-)" cells. Our preliminary results indicated that expiratory neurons were present in a higher concentration in the DRG of the guinea pig than in the DRG of the cat. Two types of "pump" units were observed. The first type was excited by lung inflation but was not driven by the central respiratory rhythm. This "pump" unit was similar to that described in the cat. The second, novel type (termed "pump-inhibited") showed tonic activity that was inhibited by lung inflation and was independent of the central respiratory rhythm. In general, the characteristics of RRUs in the DRG of the guinea pig appeared similar to those of the cat with the addition of a new type of pump related unit. (Supported by NS15350).

335.12 NEURONAL DISCHARGE PATTERNS IN THE CENTRAL NUCLEUS OF THE AMYGDALA DURING SLEEP-WAKING STATES, J. X. Zhang*, R. M. Harper and R. C. Frysinger (SPON: J. Engel). Dept. of Anatomy and the Brain Research Institute, UCLA, Los Angeles, CA 90024.

The central nucleus of the amygdala (ACE) has large reciprocal projections to the parabrachial pons, a brain stem area implicated in respiratory cycle timing and cardiovascular control. Repetitive single pulse stimulation to the ACE can pace the respiratory cycle, an effect which is sleep state dependent. High frequency train stimulation causes a rapid blood pressure rise which is accompanied by substantial bradycardia. This study examined the possibility that discharge from neurons in the ACE is related to timing of the respiratory cycle and to cardiovascular changes.

Bundles of fine wire microelectrodes were placed in the ACE of cats. After surgical recovery, we recorded neuronal discharge, diaphragmatic EMG activity, EKG and EEG from the intact, unrestrained, drug-free cat during waking and quiet and active sleep states. A set of ACE neurons demonstrated a relationship with the respiratory or cardiac cycle, as indicated by cross correlation techniques. These correlations were greatly affected by state, with the neurons demonstrating more dependency during waking, and less during quiet sleep and REM sleep. In addition to examining the interactions between ACE neurons and cardiorespiratory events, interactions between simultaneously recorded ACE neurons were studied. Cross correlations between neurons of one ACE with neurons of the contralateral ACE suggest that a proportion of these neurons were driven by a common input. These dependencies were predominantly excitatory, i.e., discharge of one cell enhanced the probability of discharge of a neighboring cell, but inhibitory cross correlations were occasionally obtained.

These results support the hypothesis that the ACE plays a role in respiratory cycle timing and cardiovascular regulation and that this influence is state-related.
Supported by HL 22418-05 and AHA-LA 678-lG2.

335.13 RESPIRATORY INHIBITION FOLLOWING HYPERTENSION DURING ANESTHESIA AND DURING SLEEP-WAKING STATES. R.B. Trelease*, G.C. Sieck, and R.M. Harper (SPON: E. Zimmermann). Department of Anatomy and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Blood pressure elevation or direct baroreceptor stimulation evokes respiratory inhibition in anesthetized animals. This inhibition includes slowing of respiratory rate, reduction of phrenic nerve discharge, alteration of bronchomotor tone, and inhibition of diaphragmatic discharge. It has been suggested that slowing of respiratory rate (increase in T_{tot}) is achieved by proportional increases in inspiratory duration (T_i) and in expiratory duration (T_e), such that the effective "duty cycle" (T_i/T_{tot}) remains relatively constant across a given range of systemic blood pressure. The present study examined whether such relationships occur in the drug-free cat and also assessed the respiratory response to elevated blood pressure in different sleep-waking states. Transient hypertension was induced in chronically instrumented, drug-free cats during sleep and waking states. For comparison, transient hypertension was also induced in tracheostomized, pentobarbital anesthetized cats, instrumented for measurement of blood pressure, diaphragmatic EMG, airflow, thoracic movement, and expired CO_2 concentrations. With blood pressure elevation, there was an increase in T_{tot} and a decrease in T_i during all states in the intact animals. There was also a decrease in the amplitude of diaphragmatic EMG activity. Blood pressure elevation thus resulted in a decreased T_i/T_{tot}. There appeared to be no marked differences between state in the basic pattern of respiratory reponse to hypertension. Occasional extreme responses, such as apneic periods, occurred predominantly during sleep states. A decrease in effective "duty cycle" (i.e., the shortening of inspiration relative to the lengthening of overall respiratory cycle duration) was also observed in anesthetized animals, as well as reductions in inspiratory airflow and tidal volume. When respiratory activity was increased by hypercapnea (5% CO_2 in O_2 inspired mixture) the inhibition resulting from transient hypertension was more pronounced. The inhibitory effects of hypertension persisted after bilateral vagotomy. These results suggest that hypertension-related slowing of respiration during sleep, waking, and barbiturate anesthesia results from a lengthening of expiratory duration, primarily by an increase in the post-expiratory pause preceding the onset of inspiration. This may be considered as an inhibition of mechanisms triggering inspiration. Furthermore, the relative reduction in inspiratory duration is consistent with demonstrated inhibition of phrenic and diaphragmatic discharge.
Supported by HL 22418-05 and AHA-LA Grant 678-lG2

335.14 LETHAL RESPIRATORY DISTURBANCE IN NEONATAL RATS AFTER ARTERIAL CHEMORECEPTOR DENERVATION. M. A. Hofer, Depts. of Psychiatry and Neuroscience, Albert Einstein Coll. of Med., Montefiore Med. Cntr., Bronx, NY 10467.

Premature infants with frequent episodes of apnea and atypical "periodic" respiration have raised questions about how rhythmic respiration is maintained during early development (1). Chemoreceptor deafferentation in neonatal animals has generally failed to disrupt rhythmic respiration but studies have been limited either to the anesthetized state or to section of only carotid sinus afferents or to a precocial species, such as the sheep.

We recorded respiration in unanesthetized rat pups in their home cages by impedance pneumography, using chronically implanted subcutaneous electrodes, at 4h and 24h following surgery. Carotid sinus nerve and aortic "depressor" fibers were identified and cut (S-A D) under ether anesthesia, using a high resolution operating microscope at 3-5, 8-10, 14-16 and 20-22 days postnatal age, a procedure that abolished acute respiratory responses to experimental hyperoxia and hypoxia.

In the 3 youngest groups (but not in 3 week olds) S-A D produced an episodic respiratory disturbance with apneas and periods during which the usual sine wave pattern of linked respiratory phases was replaced by an arrhythmic series of low amplitude waveforms, short pauses and occasional high amplitude gasps, often leading to cyanosis before reversion to normal respiratory rhythm occurred. In the 3-5 and 8-10 day old S-AD pups (N=16) this atypical respiration occupied 30-50% of recording time and was associated with a 50% mortality rate within 10 days of surgery, whereas pups of the same ages given control operations with identical dissections, but with chemoreceptor nerves left intact (N=12), showed normal respiratory patterns and none died.

Ongoing studies of sleep-wake state patterns in 12-14 day old S-A D pups (N=4) show that 94.5% of atypical respiration occurs during REM sleep. Tracheostomy does not appear to alter the abnormal respiratory patterns after S-AD.

These results suggest that, in this slow developing species, chemoreceptor feedback may play an unexpected role in the maintenance of rhythmic respiration during early postnatal development.

335.15 THE RESPIRATORY RESPONSE TO TRH IN THYROIDECTOMIZED RATS. R. A. Mueller, A. C. Towle and G. R. Breese. Depts. Anesthesiology, Anatomy, Pharmacology, and Psychiatry, Univ. of North Carolina at Chapel Hill, Sch. of Med., Chapel Hill, NC 27514.

The intracerebral (i.c.v.) administration of TRH is known to produce a dramatic increase in respiratory rate, minute ventilation, depression of $PaCO_2$, and an apparent displacement of the minute ventilation-$PaCO_2$ curve to the left. The site of TRH action is probably within the CNS because the doses needed after i.c.v. administration are lower than those required intra-arterially. Nonetheless, since TRH stimulates the anterior pituitary to secrete TSH and thus ultimately thyroid hormone, we examined the possibility that thyroxin release might contribute to the TRH induced respiratory changes.

Propylthiouracil administered for 10-13 days shifted the minute ventilation-$PaCO_2$ curve to the right, but the response to exogenous i.c.v. administration of TRH was similar to that in control rats. When measured at the conclusion of respiratory studies no significant changes relative to control were noted in the serotonin, 5HIAA, TRH or substance P content of the hypothalamus, pons-medulla, or midbrain in propylthiouracil treated rats.

Hypothyroidism induced by adolescent thyroidectomy reduced the slope of the minute ventilation-$PaCO_2$ curve and shifted it to the right when examined two weeks later. Supplementation with thyroxin (25µg/kg/day) restored the curve to normal except at high $PaCO_2$ levels, where it was still depressed. The minute ventilation response to exogenous i.c.v. TRH administration was delayed and of smaller absolute magnitude relative to control rats, whether or not they were supplemented with thyroxin. No significant changes in serotonin, 5HIAA, TRH or substance P were noted in the pons-medulla or hypothalamus in thyroidectomized rats when compared to control. However, in spite of calcium dietary supplementation, all thyroidectomy animals had total serum calcium values significantly below normal.

We conclude that the response to TRH is not secondary to its ability to indirectly increase thyroxin secretion. In addition, the respiratory response to TRH may well be dependent on normal calcium availability.

335.16 COMPARISON OF CENTRAL NERVOUS SYSTEM AND NEUROMUSCULAR EFFECTS OF ACUTE INFUSION OF TABUN OR VX. E.T. Beers*, R.E. Foster, J.F. Glenn, N.L. Adams*, A.V. Finger*, T.C. Randolph*, D.L. Rickett. Neurotoxicology Branch, U.S. Army Medical Research Institute of Chemical Defense, APG, MD 21010.

Tabun (GA) and VX are organophosphate anticholinesterase (anti-ChE) agents. The present study was conducted to examine the acute toxicities of GA and VX and to identify the relative significance of their actions on the central nervous system (CNS) and at the periphery.

Using Dial-urethane anesthetized cats, recordings were made of neocortical and cerebellar EEG, medullary respiratory-related units, phrenic nerve discharge electromyographic activity and contractions of the diaphram leaflet, airflow, blood pressure, electrocardiographic activity (ECG) and electroencephalographic activity (EEG). Blood gases and expired CO_2 were also monitored. The agents were administered at a rate of one ml/min in a concentration of 1 LD50/15 ml. The infusion was stopped when spontaneous respiration ceased, whereupon diaphragmatic neuromuscular blockade was tested by administration of supramaximal stimulation of the phrenic nerve (10 hz and 100 hz, 0.5 msec pulse, 0.5 sec duration) and artificial respiration was initiated. Agent infusion was then continued at the previous rate but at three times the initial concentration, and diaphragmatic responsiveness was tested at varying intervals.

Both GA and VX were similar in effect to soman (GD) and sarin (GB), two anti-ChE agents studied previously. The results show that there was a loss of synchronous discharge of respiratory-related units. This disruption of normal neuronal activity preceeded agent-induced respiratory arrest and was maximally reflected as a loss of phrenic nerve discharge. Prior to ceasation of spontaneous respiration, the ECG showed loss or inversion of the "t" wave and cerebral electrical activity began producing high voltage, synchronous spikes of 5-7 hz frequency. At the time that spontaneous respiration ceased, contractions of the diaphram could be elicited by phrenic nerve stimulation. It was not possible to establish the dose of GA or VX required to block the diaphram, since in most cases cardiac failure and loss of blood pressure precluded further infusion of the agents. The findings suggest that GA and VX toxicity, like GB and GD toxicity, is acutely mediated through a loss of central respiratory drive; this results from disruptions of normal patterned activity in respiratory-related unit discharge. GA and VX also appear to be more cardiotoxic than soman. Our findings also suggest that areas of the cerebellum, as well as the cerebrum, are sensitive to agent-induced seizures, although further studies are required to quantify these observations.

335.17 QUANTIFICATION OF SUCCINATE DEHYDROGENASE ACTIVITY OF DIAPHRAGMATIC MUSCLE FIBERS. G.C. Sieck, R.D. Sacks,* C.E. Blanco* and V.R. Edgerton. Dept. Respiratory Diseases, City of Hope Medical Center, Duarte, CA 91010, Depts. Anatomy and Kinnesiology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Histochemical characterization of 2 types of fast-twitch muscle fibers in skeletal muscle has been based primarily on their oxidative capacity. Fast-twitch glycolytic (FG) fibers are characterized as having lower oxidative capacity than fast-twitch oxidative glycolytic (FOG) fibers. Fast-twitch motor units have also been classified into 2 types based on their susceptibility to fatigue. Thus, a correlation has been drawn between the fatigue resistance of a motor unit and the oxidative capacity of its constituent muscle fibers. In the cat diaphragm, we determined the oxidative capacity of muscle fibers using quantitative image processing techniques. Diaphragms from 5 cats were excised and segments were taken from the costal, crural and crossing-band regions of the muscle. Serial cross-sections from each region were stained for succinate dehydrogenase (SDH) activity. Individual muscle fibers within the section were then delineated and their optical staining density for SDH was determined. Initially, the linearity of the SDH reaction was verified by taking repeated scans at 1 min intervals as the reaction developed. Once linearity was established, an end-point of 8 min was selected for subsequent determinations of the rate of the SDH enzymatic reaction. Fiber samples from each diaphragmatic region were taken from across the entire abdominal to thoracic extent of the muscle. Approximately 60% of the sampled fibers were characterized as fast-twitch and 40% as slow-twitch based on myosin ATPase activity. Fast-twitch fibers in the diaphragm did not divide into 2 populations based on their SDH activity, i.e., one group with high oxidative capacity (FOG) and another with low oxidative capacity (FG). Instead, SDH optical staining densities of fast-twitch fibers showed a unimodal distribution that was skewed toward lower SDH enzymatic rates. Yet, of all the fast-twitch fibers sampled, approximately 40 to 50% had SDH activity that overlapped that of slow-twitch fibers. Since fast-twitch motor units are readily divisible into 2 types based on physiologic differences, these results question whether there is a strong correlation between the oxidative capacity of fast-twitch muscle fibers and the fatigue resistance of fast-twitch motor units.
Supported by NIH grants NS16333 and HL29991.

336 SYMPOSIUM. THE OCULOMOTOR ROLE OF THE ACCESSORY OPTIC SYSTEM AND PRETECTUM: IS THERE A SINGLE VERTEBRATE SCHEME?

J. Wallman, City Univ. of New York (Chairman); K.V.Fite, Univ. Massachusetts; J.I. Simpson, New York Univ. School of Medicine; K.-P. Hoffmann, Universitaet Ulm; J.T. Weber, Tulane Univ.

After decades of obscurity as visual pathways of unknown function, the projections to and from the accessory optic system (AOS) and pretectum (PT) have recently excited considerable interest, in part because of intriguing anatomical evidence of projections from these retinorecipient regions to the cerebellum and the oculomotor nuclei, and in part because of physiological and behavioral evidence that these nuclei are the brain's major source of information on large-field movements of the visual surround (the so-called retinal slip signal). This signal is used in a number of different ways by the visual and oculomotor systems, of which visually guided compensatory eye movements (optokinetic responses) have been most extensively studied and most clearly depend on the AOS and PT in a variety of species. The simplest view is that directionally selective retinal ganglion cells project to the AOS and PT where the output of many neurons are combined into a large-field retinal slip signal. This signal somehow gets to the vestibular nuclei (as well as the cerebellum), where it is combined with signals from the semicircular canals, and then goes to the motor nuclei producing the compensatory eye movements.

A major question concerns the extent to which the neural organization of this system is basically the same in all vertebrates. Although much evidence argues for a single vertebrate pattern of organization of AOS and PT, some important and provocative differences have emerged from recent work. This symposium will seek to determine whether these differences reflect fundamental species differences in the organization of this neural system. Specifically, we will consider the following questions: (1) Which anatomical classes of retinal ganglion cells project to the AOS and PT in different species? (2) Do neurons in the AOS or PT send a direct projection to the oculomotor nuclei? (3) Does the AOS project directly to the cerebellum? (4) What are the response characteristics of AOS and PT neurons, and can these characteristics be related to those of optokinetic behavior, either in terms of directional or velocity preferences or in terms of binocularity? (5) Is there evidence for non-visual signals being carried by PT or AOS neurons? (6) What role does the visual cortex appear to play in the neuronal responses of the AOS and PT, as well as in optokinetic behavior? (7) What differences are there in the roles played by the AOS and PT in optokinetic behavior?

337

ABSTRACT 337 NOT AVAILABLE

COMPARATIVE NEUROANATOMY II

338.1 NEUROMODULATORS AND NEUROTRANSMITTERS IN THE MOLLUSKAN NERVOUS SYSTEM. S.C. Feldman. Dept. of Anatomy, U.M.D.N.J.-New Jersey Medical School, Newark, N.J. 07103.

Immunohistochemical analysis of the distribution of transmitter/modulator systems in the mammalian CNS suggests that there are three basic patterns of organization: systems such as the biogenic amines whose neurons are restricted to a small number of nuclei and have extensive fiber systems; systems such as somatostatin (SRIF) whose cell bodies are present in most areas of the CNS in a variety of neuronal cell types; and the majority of neuropeptide systems whose cell bodies and fibers are found in several, but not the majority, of CNS nuclei. The rationale for such differences in molecular organization are not clear; we have begun a comparative study of the distribution of transmitter/modulator systems in invertebrates. In this report we describe the distribution of serotonin (5-HT), SRIF, and Substance P (SP)-containing neurons and processes in the nervous system of the squid, Loligo pealeii.

Squid were collected at Marine Biological Laboratory, Woods Hole; tissue was fixed in Bouin's solution and immunocytochemistry carried out on either 50-100 um Vibratome, or 6-12 um paraffin-embedded, sections. Primary antisera to 5-HT and SP were obtained from Immunonuclear Corp.; antiserum to SRIF was a gift from Dr. E. Lichtenstein and has been used extensively by us. All staining could be blocked by prior adsorption of the antiserum with the appropriate antigen; endogenous peroxidase activity was minimal.

In general, the distribution of all three molecules was more restricted in the squid nervous system than in mammals. Immunoreactive 5-HT and SRIF neurons were seen only in the medulla of the optic lobe and two lobes of the supraesophageal complex; SP neurons were found only in one lobe of the supraesophageal ganglion and scattered neurons in the stellate ganglion. For all three transmitters the immunoreactive perikarya were either small or medium-sized; there were no immunoreactive large neurons, although some appeared to receive a SRIF innervation. In the optic lobe, both SRIF and 5-HT were present in the larger neurons between cell islands and in the small, possibly axonless, neurons; 5-HT and SRIF fibers were present in the plexiform layer, the neuropil of the medulla and the optic tract. The subesophageal ganglia, which had no immunoreactive neurons, received an extensive SRIF and 5-HT innervation. The results of this study suggest that the restrictive pattern of neuronal transmitter distribution seen for 5-HT and SP in the mammalian CNS may be phylogenetically very old; the widespread distribution of SRIF in mammals may suggest an expansion of function with evolution. It should be recalled, however, that the squid nervous system is extremely complex; examination of transmitter distribution in less complex organisms may help to resolve this issue.

338.2 INDOLEAMINE UPTAKE IN TECTAL NEURONES OF THE FROG. D. Bieger & R.S. Neuman, Faculty of Medicine, Memorial University, St. John's, Newfoundland, Canada A1B 3V6.

Among various sensory structures of the frog brain previously reported to receive a serotoninergic innervation, visual receptive areas are prominently represented, including lateral geniculate nucleus, pretectal region, optic tectum and nucleus isthmi. Evidence available from histofluorescence microscopical studies suggests that serotoninergic afferents to these regions originate from perikarya located in the brainstem paramedian zone. While utilizing the neurotoxic serotonin (5HT) analogue, 5,7-dihydroxytryptamine (5,7DHT), for the visualization of 5HT neurones in Rana pipiens brains, we confirmed that, following intraventricular injections in vivo, this compound is taken up by raphe cells as evidenced by the appearance of a characteristic bluish-white histofluorescence. In addition, however, a discrete population of tectal perikarya located in layers 6 and 8, and resembling large pear-shaped neurones of Scalia, were also seen to develop specific fluorescence. Moreover, practically identical results were obtained when the uptake of 5,7DHT was examined in vitro, using isolated hemisected brain preparations maintained in an oxygenated Ringer's medium. In further studies with the in vitro preparations, we tested for possible uptake of several other indoleamines. Evidence of uptake was obtained with 5-HT and 6-HT, whereas N-acetyl serotonin and melatonin did not appear to be accumulated by the perikarya in question. Competition between 5-HT and 5,7DHT for uptake could be demonstrated; however, attempts to block uptake of 5,7DHT by impramine, ouabain or low temperature failed. Apart from allowing extensive visualization of dendritic processes of the tectal perikarya, 5,7DHT-labelling also permitted tracing of two tectofugal fibre pathways coursing between tectal layer 7, on the one hand, and varicose terminal fields in the pretectal area and nucleus isthmi, on the other. The latter output may originate from tectal neurones receiving binocular input. Although earlier histofluorescence and recent immunohistochemical studies have failed to reveal serotonin perikarya in the frog optic tectum, our observations indicate that this brain region contains neurones possessing striking capability for accumulating indoleamines. To our knowledge, this is the first report of a selective uptake of serotonin into what appear to be vertebrate non-monoaminergic neurones.

Supported by MRC-Canada

338.3 NUCLEUS ISTHMI IN THE FROG: CONNECTIONS WITH MONOCULAR TECTUM. P. Grobstein and T. Masino*. Dept. Pharm. Physiol. Sci., Univ. of Chicago, Chicago, Ill. 60637

Each nucleus isthmi in Rana pipiens receives a projection from the ipsilateral and sends a projection to the contralateral tectal lobe. Prior work on the nucleus as an intertectal relay focused largely on projections related to binocular tectal regions, regions representing areas of visual space mapped in both tectal lobes. We have studied the projections related to monocular tectal regions, regions representing areas of visual space mapped in only one tectal lobe. Projections were analyzed from retrograde and anterograde labelling patterns following small injections of HRP into electrophysiologically characterized tectal loci.

Afferents from monocular tectum project to the dorsal half of the nucleus. Medial loci project more medially, lateral loci more laterally, rostral loci more ventrally, and caudal loci more dorsally. Afferents terminate through the rostro-caudal thickness of the nucleus, coming into proximity to the cells, also dorsally located, which project to the opposite monocular tectum. The topographic organization of the crossed efferent projection is like that of the afferent projection. Medial regions project medially, lateral regions laterally, ventral regions more rostrally, and dorsal regions more caudally.

The afferent projection from monocular tectum is topographically continuous with that from binocular tectum. In contrast, the monocular component of the crossed efferent projection is not continuous with the binocular component and differs in the direction of the mapping along one axis. Nearby cells at the middle of the nucleus project to quite different locations in the opposite tectal lobe. The more dorsal cells project to rostral monocular and the more ventral cells to rostral binocular tectum. Caudal in monocular tectum corresponds to dorsal in the nucleus while caudal in binocular tectum corresponds to ventral.

These results suggest that monocular, like binocular, tectal regions may be linked by an intertectal pathway through the nucleus isthmi. Ventrally, afferents from binocular tectum are associated with cells projecting to the opposite binocular tectum. This efferent projection is inverted around one axis with respect to the afferent projection, yielding a pattern of intertectal connectivity linking tectal loci which, rather than being symmetrically located, relate to the same direction in visual space. The efferent projection from the dorsal region related to monocular tectum is not inverted. The resulting intertectal connectivity pattern is appropriate to link symmetrically located tectal loci, loci which receive input from mirror symmetric locations in visual space. Whether this pattern is physiologically significant or a remnant of developmental events involved in establishing the binocular pattern of intertectal connectivity remains to be determined.

Support by PHS EY-01658 and NSF BNS-7914122

338.4 The organization of retinal projections to the diencephalon in the teleost fish Haplochromis burtoni. J. Presson, R. Fernald, and M. Max*. Institute of Neuroscience, University of Oregon, Eugene, Oregon, 97403.

The retina in teleost fish sends a large projection to the optic tectum and several smaller projections to the diencephalon. The diencephalon contains at least 5 cell groups that receive retinal input (Fernald, JCN, 1982). Based upon a current organizational scheme (Bradford and Northcutt, Fish Neurobiology, in press) the visual diencephalon can be divided into 3 general regions: the preoptic, the pretectal and the dorsal and ventral thalamic. These teleost structures may be functional counterparts to nuclei in the mammalian diencephalon, but the assignment of functions to the various teleost cell groups is not yet possible. In order to add to our knowledge of the teleost visual diencephalon we have studied the details of the retinal projections to these structures. The experiments are designed to reveal any differential inputs from various retinal regions and any retinotopic organization in the projections. The teleost species that we have studied is the highly visual, African cichlid fish Haplochromis burtoni. Small cuts in the retina were filled with pellets of HRP and the brains were processed with either the Hanker-Yates or TMB reactants.

A retinal projection to the caudal preoptic region of teleosts has been reported by many investigators. This region is assumed to be functionally equated with the suprachiasmatic nucleus in mammals. In H. burtoni, the brains processed with Hanker-Yates revealed only a very small projection to this region. The projection appears to arise from only dorsal retina. Experiments with the more sensitive TMB method will be reported in an attempt to more accurately characterize this projection.

The most prominent pretectal nucleus is the superficial pretectal. This structure lies laterally in the brain, just rostral to the optic tectum, and has sometimes been called the lateral geniculate. It consists of a folded sheet of cells surrounding a central neuropil. Retinal cuts placed at various angles from the falciform process indicate that the superficial pretectal receives input from all retinal quadrants and that the input is organized retinotopically. Temporal retina projects rostrally, nasal retina projects centrally, dorsal retina projects caudomedially, and ventral retina projects caudolaterally.

The ventral and dorsal thalamic region is comprised of several cell groups. As a whole this region receives input from all retinal quadrants. The inputs to individual cell groups, as well as the degree of retinotopy in the projection, will be presented in more detail. Supported by the Whitehall Foundation.

338.5 PERIPHERAL DISTRIBUTION AND PRIMARY PROJECTIONS OF THE LATERALIS NERVES IN THE GOLDFISH, CARASSIUS AURATUS. Richard L. Puzdrowski*. (SPON: M. S. Northcutt). Division of Biological Sciences, University of Michigan, Ann Arbor, Michigan 48109.

The peripheral distribution of the lateralis nerves was examined using the Williams ('43) modification of the Sihler technique. The central projections of these nerves were traced with HRP. Adult goldfish (8-12 cm) were anesthetized with MS222 and individual branches of the anterior (ALLN) or posterior (PLLN) lateral line nerve were exposed and transected. A gelfoam pledget soaked with 40% HRP (Sigma VI) was placed on the proximal stump of the transected branch. After a survival time of 7-11 days at 22-26°C, the animals were reanesthetized and transcardially perfused with phosphate buffer (pH 7.4) followed by a 2% glutaraldehyde solution. The brains were transversally or horizontally sectioned at 40μ and processed according to the Mesulam ('78) TMB protocol, or by the Hanker-Yates protocol.

Branches of the ALLN were found in the supraorbital, infraorbital and the hyomandibular nerves. The PLLN consists of dorsal and ventral trunk branches and a supraoccipital branch. Both trunk branches are associated with a ramus of the sensory branch of the VIIth nerve.

The ALLN of the goldfish possesses a dorsal and a ventral root. Both roots enter the medial octavolateralis nucleus (MON) just caudal and dorsal to the sensory root of VII. Upon entering, the fibers of the ALLN form ascending and descending bundles. The bulk of the fibers terminate within the ventral portion of the MON. The branches of the ALLN were found to map somatotopically on the MON. A portion of the ascending fibers continue rostrally to terminate in the anterior portion of the ipsilateral eminentia granularis. Additionally, a portion of the descending fibers continue caudally to terminate in the ipsilateral caudal octavolateralis nucleus.

The PLLN enters the medulla as a single root and projects the entire extent of the MON dorsal to the terminating fibers of the ALLN. Some of the PLLN afferents continue rostrally to terminate in the posterior portion of the ipsilateral eminentia granularis. Projections to the ipsilateral caudal nucleus were also observed.

Efferent cells were found in the diencephalon and medulla following ipsilateral filling of any individual branch of either lateral line nerve. The efferents found in the diencephalon are located in the zona limitans. The medullary efferent somata are located dorsal to the medial reticular formation in close association with the Mauthner axons.

(Supported in part by NIH grants NS11006 and EY02485)

338.6 SOME CONNECTIONS OF THE TELENCEPHALON IN THE OSTEOGLOSSOMORPH TELEOST, XENOMYSTUS NIGRI. Mark R. Braford, Jr. Dept. of Anatomy, Georgetown Univ. Sch. of Med. and Dent., Washington, D.C. 20007.

The organization of the telecephalon of the teleost fishes appears to differ considerably from that of the land vertebrates. In order to compare telencephalic organization in these two large groups of vertebrates, a detailed analysis of the connectivity patterns of telencephalon in several teleosts is currently being undertaken.

A series of specimens of Xenomystus nigri (family: Notopteridae) were subjected to horseradish peroxidase (HRP) injections in one of the following brain regions: olfactory bulb, various portions of the telencephalon proper, optic tectum, torus semicircularis, and midbrain tegmentum. Following olfactory bulb injections, HRP-labeled fibers and terminals were seen in several regions of the area ventralis of the telencephalon and a large ventrolaterally located region of the posterior area dorsalis (Dp). In separate sets of experiments, HRP was injected into the medial (Dm + Dc-m) or lateral (Dd + Dc-d) regions of the posterior area dorsalis, which do not receive input from the olfactory bulb. Following medial injections a large number of cells were retrogradely labeled in certain portions of the "preglomerular" nuclei of the basal diencephalon. In separate HRP experiments, some of these cells were shown to receive a projection from the torus semicircularis. Lateral injections retrogradely labeled a different contingent of "preglomerular" cells which lie dorsal to those mentioned above and anterogradely labeled a remarkably large fiber bundle which descends along the ventral surface of the hypothalamus before making a dramatic dorsomedial sweep into the rostral midbrain tegmentum. It terminates densely in a periventricular cell group which lies slightly caudal to the oculomotor nucleus. HRP injections in this region of the tegmentum retrogradely labeled large cells in Dc-d. Other populations of Dc cells were labeled after tectal, toral, and olfactory bulb injections. More ventrolateral telencephalic HRP injections which involved Dl resulted in massive labeling of anterior commissural fibers and of terminals and cells in the contralateral telencephalon. These injections also retrogradely labeled yet another contingent of "preglomerular" cells which lies most caudolaterally in the complex. More rostromedial telencephalic HRP injections resulted in a very dense labeling of fibers which terminate in the caudal hypothalamus. Following a number of the telencephalic injections, HRP-labeling was seen in cells of the superior raphe nucleus, a group of large scattered cells in the midbrain tegmentum at the level of the interpeduncular nucleus, and cells of the locus coeruleus.

(This work was supported by NSF Grant BNS 8118844.)

338.7 SEGREGATION OF ELECTRORECEPTIVE AND MECHANORECEPTIVE LATERALIS AFFERENTS IN THE CHONDROSTEAN HINDBRAIN, J.G. New and D. Bodznick, Dept. of Biology, Wesleyan Univ., Middletown, Conn. 06457

Sensory input from the lateral line system enters the hindbrain of chondrostean fishes via an Anterior (ALLN) and Posterior Lateral Line Nerve (PLLN). The ALLN projects to the Dorsal Nucleus (DN) of the medulla via a dorsal root, and to the Medial (MN) and Caudal Nuclei (CN) via a ventral root and consists of afferent fibres from peripheral electro- and mechanoreceptors. The PLLN consists of afferent fibres from trunk mechanoreceptors and enters the MN as a single root. Averaged evoked potential, single and multiple unit activities, as well as experimental anatomical results were studied in order to determine the distribution of ALLN afferents.

Juvenile specimens of Shovelnose, Scaphirhynchus platorynchus, and Atlantic Sturgeon, Acipenser oxyrhynchus, and Paddlefish, Polyodon spathula were anesthetized with MS-222 during surgery to expose the brain and then immobilized with curare (1mg/kg). Recordings were made with a glass micropipette electrode of 8-10μM tip filled with 2M NaCl and fast green dye for marking recording sites. In some specimens a pledget of gelfoam saturated with HRP was implanted subcutaneously beneath a small petch of ampullary electroreceptors distal to the lateral line. After survival times of 14-20 days the brains were removed, sectioned, and processed to reveal fibre projections from localized ampullae.

In response to presentation of a uniform electric field of 100 μV/cm,descending electrode tracks through DN yielded large evoked potentials which decreased ventral to DN. Direct electrical stimulation of the PLLN resulted in EPs that were maximal within the MN. Single and multiple units recorded in DN responded to electrical fields, but not to PLLN or mechanosensory stimuli such as water displacement. In contrast, units recorded in MN responded to mechanical stimuli, but not to electric fields. Neuronal transport of HRP revealed fibres innervating peripheral electroreceptors projecting only to DN via the dorsal root of the ALLN.

Thus it appears that electroreceptive afferents of the ALLN project to DN via the dorsal root of ALLN, and mechanoreceptive afferents project to MN via the ventral root of ALLN and the PLLN.

338.8 CENTRAL PROJECTIONS OF TRIGEMINAL AND LATERAL LINE NERVES IN THE BLIND CAVE FISH, *ASTYANAX HUBBSI*. Theodore J. Voneida, S. E. Fish and T. C. Cauller*. N.E. Ohio Univ. College of Medicine, Neurobiology Program, St. Rt. 44, Rootstown, Ohio 44272.

Behavior of blind cave fish is known to be strongly dependent on tactile and vibratory cues. Our studies have shown that the cave fish tectum is unresponsive to visual or vibratory stimuli, but is highly responsive to somatosensory stimuli. The torus semicircularis is known to be responsive to vibratory stimuli in all animals studied (including our observations on blind fish). This study examines central projections of trigeminal and lateral line nerves in blind fish, and relates the results to those obtained from a parallel study of tectal and toral afferents in cave fish. Central connections of lateral line; supra- and infraorbital branches of the trigeminal nerves were examined by unilateral placement of pledgets saturated with 40% HRP on cut ends of each nerve. Trigeminal branches were cut intraorbitally; the descending lateral line nerve was cut immediately caudal to the gill. Following survival times of 5-18 days at 25-30° C, animals were perfused transcardially, the brain removed, embedded, serially sectioned and reacted with TMB to visualize anterograde transport of HRP. Descending lateral line projections correspond closely to results described by others; namely, a dorsal fascicle terminating in nucleus medialis and emenentia granularis, and a caudal projection into an area which appears to correspond to nucleus caudalis. As might be expected, trigeminal nerve dips resulted in projections to these same areas, since lateral line nerves to the head are known to travel in both branches of nerve V. This fits with our finding (reported at this meeting) that these same nuclei project directly to the lateral nucleus of the torus semicircularis, and suggests that a monosynaptic connection exists between lateral line structures of the head and the torus. Trigeminal dips also labeled the spinal tract of V, from which ventromedial branches were traced into a longitudinal column of cells corresponding to the nucleus descendens of other authors. This continues caudally into the upper cervical cord, in a position corresponding to Herrick's ('06) medial funicular nucleus. We also identified a medial projection from the spinal tract to a region ventral to the Motor X nucleus, as described by Luiten ('75) in carp. The fact that primary trigeminal axons terminate on cells in which we have found retrograde label following tectal injections supports our physiologic studies, in which the cave fish tectum was found to be highly responsive to somatosensory stimuli from the head. In addition, it suggests that this response is mediated via a monosynaptic connection between primary trigeminal axons and brainstem nuclei which project directly to the tectum.

This work was supported by NIH Grant NS18369.

338.9 THE ORGANIZATION OF THE MOTORNEURONS INNERVATING THE AXIAL MUSCULATURE OF GOLDFISH. J.R. Fetcho* (Spon: L. T. Rutledge). Div. Biological Sciences, U. Michigan, Ann Arbor MI. 48109.

Axial muscles are important in vertebrate locomotion. The axial musculature of all vertebrates develops as a series of repeated muscle blocks, or myotomes, and in most vertebrates the myotomal arrangement persists as a series of myomeres in the adult animal. In aquatic anamniotic vertebrates the myomeres are usually divided into superficial red and deep white portions.

A study of the motorneurons innervating the axial musculature of the goldfish was undertaken: 1.) to investigate whether the spatially patterned innervation of muscle masses established for the limb muscles (Landmesser, J.Physiol. 284:371; Hollyday, J.C.N. 194:143) is also a feature of the organization of axial muscle motor nuclei, and is thus a general feature of somatic motor systems, and 2.) to investigate features of motorneuron organization that might relate to known functional differences between the superficial red and deep white portions of fish axial muscle.

HRP was applied to the dorsal portion of the myomeres of the tail on one side of goldfish, and to the ventral portion of the myomeres on the opposite side of the same fish to compare the positions and sizes of motorneurons innervating the dorsal and ventral extremes of the myomeres. After four to six days the spinal cords were removed, serially sectioned and reacted with cobalt/DAB. There were no differences in the sizes or positions of dorsal and ventral myomeric motorneurons in the medial motor column (MMC), implying there is no correspondence between dorso-ventral position in the myomere and position of motorneurons in the MMC.

HRP applied to both superficial red and deep white myomeric muscle resulted in labeled motorneurons throughout the transverse extent of the MMC, while HRP applied to only red muscle resulted in labeled motorneurons confined to, or concentrated in, the ventral portion. The size distribution of labeled red plus white muscle motorneurons was skewed, with a large population of smaller motorneurons and a "tail" of larger motorneurons. Labeled superficial red muscle motorneurons generally occupied the lower end of the size distribution typical of red plus white muscle labeling. HRP applied to white muscle alone resulted in filled motorneurons throughout the size range typical of red plus white muscle labeling, but without the large population of smaller motorneurons characteristic of red muscle labeling. These experiments indicate the white muscle is innervated by large motorneurons not innervating red muscle, and that some of the motorneurons innervating red muscle do not innervate white muscle. The differences in the size distributions and locations of superficial red and deep white muscle motorneurons may be related to the primary role of white muscle in rapid locomotion of fish. (Supported by an NSF Predoctoral Fellowship to J.R.F. and EY00168 to S.S. Easter, Jr.)

338.10 THE PRIMARY LATERAL LINE AFFERENTS IN LEPIDOSIRENID LUNGFISHES. R. Glenn Northcutt. Division of Biological Sciences, University of Michigan, Ann Arbor, Michigan 48109.

The peripheral course and types of receptors innervated by the anterior and posterior lateral line nerves in African (Protopterus) and South American (Lepidosiren) lungfishes was determined by gross dissection and histological examination of juvenile and adult specimens. Lepidosirenid lungfishes possess both ordinary mechanoreceptive neuromasts and electroreceptive ampullary organs (organs of Fahrenholz). Histological examination of skin samples reveals that ampullary organs in these lungfishes are found on the trunk, as well as on the head as in most other primitive fishes.

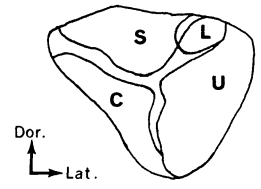
The anterior lateral line nerve (ALLN) possesses supraorbital, infraorbital, mandibular, and hyomandibular rami that innervate head receptors, as in other fishes, but the ALLN differs in also possessing a recurrent otic ramus that passes laterally and caudally around the otic capsule to give rise to three trunk rami (dorsal, lateral, and ventral) that innervate all ampullary organs of the trunk. The posterior lateral line nerve (PLLN) also possesses three rami (dorsal, lateral, and ventral) that course along with the corresponding trunk branches of the ALLN; however, the branches of the PLLN innervate only trunk neuromasts.

Various branches of the ALLN and PLLN were unilaterally transected, and plegets of gelfoam saturated with HRP (Sigma VI) were applied to the proximal stumps in anesthetized juvenile specimens of Protopterus and Lepidosiren. Following survival times of 8-10 days at 23-26°C, the animals were reanesthetized and perfused. The brains and the proximal portions of the cranial nerves were reacted with tetramethyl benzidine or Hanker-Yates reagent to visualize the central projections of the lateral line nerves. HRP-labeling of the ALLN rami restricted to the head, or labeling of trunk lateral line rami, resulted in primary fibers terminating in both the ipsilateral dorsal and medial octavolateralis nuclei and eminentia granularis, whereas HRP-labeling of the recurrent otic ramus of the ALLN resulted in primary fibers terminating only in the ipsilateral dorsal octavolateralis nucleus (DON). The lateral line fibers terminate somatotopically within DON, so that the head is represented rostrally and medially and the trunk laterally and caudally. A similar somatotopy exists within the terminal neuropil of the medial octavolateralis nucleus (MON). However, within the MON, the entering mechanoreceptive fibers of both lateral line nerves form dorsal and ventral fascicles.

(Supported in part by NIH grants NS11006 and EY02485)

338.11 CENTRAL AFFERENT CONNECTIONS OF THE MACULAE OF THE INNER EAR OF THE CLEARNOSE SKATE, RAJA EGLANTERIA. Michael A. Barry. School of Life and Health Sciences, Univ. of Delaware, Newark, DE 19711.

The central projections of afferents innervating the maculae of the sacculus, lagena, and utriculus of the skate were revealed following the transganglionic transport of HRP or HRP-WGA conjugate from individual eighth nerve branches. The octavolateralis area of the medulla is divided into three longitudinal columns of cells and neuropil; namely, a dorsal electroreceptive lateral line lobe, an intermediate mechanoreceptive lateral line lobe (nucleus intermedius), and a ventral octaval column which receives first order octaval afferents. The ventral column consists of four nuclei: ascending (AON), magnocellular (MON), descending (DON), and posterior (PON). Each macula projects to PON, probably to MON, and to limited and largely nonoverlapping areas within DON and AON (see diagram of transverse section through AON). Within both AON and DON, saccular afferents course and terminate in dorso-medial areas (S), utricular afferents in the lateral half of the nuclei (U), lagenar afferents in a dorso-lateral area (L) between utricular and saccular zones, and the remainder of eighth nerve fibers (from canal organs and the macula neglecta) in ventral parts (C) of the nuclei. Afferents from medial parts of the saccular macula tend to distribute medially within the saccular zone of AON and DON.



Although all macular sense organs may be multisensory, the saccular macula, macula neglecta, and lacinia of the utricle are suspsected to serve as auditory receptors in skates. Each of the four primary octaval nuclei receives afferents from each macula and from the semicircular canal cristae; therefore, it is unlikely that any of the four nuclei subserve only an auditory function. An anatomical separation of auditory and vestibular functions must occur within one or more of the four nuclei. Primary afferents of the entire eighth nerve also course to parts of the reticular formation, nucleus intermedius, and the pars lateralis and medialis of the vestibulocerebellum. However, afferents from the three maculae differ from those of the entire eighth nerve (and thus from canal afferents), since they project very sparsely to the pars medialis of the vestibulocerebellum, and, except for utricular afferents, to the reticular formation. Supported by USPS Grant NS11272 to R. L. Boord.

338.12 RELATIVE BRAIN SIZE IN PRIMATES. E. Armstrong* (SPON: B.V. Updyke). Dept. of Anatomy, L.S.U. Med. Ctr., New Orleans, La. 70112.

The brain size relative to body weight of prosimian primates resemble those of non-primate terrestrial mammals. Anthropoids (monkeys, apes, humans), on the other hand, have larger brain-to-body weights and this is reflected in higher encephalization indices (EI). Recently it has been shown that mammalian brain weights (E) vary isometrically (as a simple ratio) with its energy supply; a factor approximated by body weight (S) and oxygen turnover or basal metabolic rates(BMR). The isometric relationship is thought to be the result of the continuous metabolic needs of the brain (Armstrong, Neurosci. Lett. 34: 101, 1982). Differences between nonhuman anthropoid and prosimian relative brain sizes may stem in part from low prosimian BMR's.

To test this hypothesis, brain and body weights and BMR's were collected from published reports and analyzed allometrically. If the body weights of the individuals of a species used in the brain-body weight and BMR-body weight studies differed by more than 10%, the BMR's were adjusted by: $\log BMR_2 = 0.25 (\log S_1 - \log S_2) + \log BMR_1$.

The encephalization indices were determined from the equation $\log E = -1.23 + .76 \log S$ which describes the empirical brain-body relationships among 93 mammals (Armstrong, Science, in press). Prosimian EI's (N=7) were significantly lower than anthropoid (N=13) EI's ($t_{(18)} = 2.45$, $p<.05$). Adjusted encephalization indices (AEI) take into account BMR's and are based on the equation $\log E = -2.11 + 1.026 \log(S \times BMR)$ (Ibid). In this case, prosimian relative brain sizes cannot be distinguished from nonhuman anthropoid ones ($t_{(18)} = 1.147$, $p > .05$). Primates differ from other mammals in their AEI's ($t_{(91)} = 2.2$, $p<.05$). The high AEI's in extant prosimians and anthropoids and the differences separating these primates from other mammals suggests that a relatively enlarged brain for the organism's energy supply was an early primate adaptation and that an increase in BMR was a preadaptation for anthropoid brain-body relationships. Supported in part by NSF B.N.S.-8204480.

BRAIN METABOLISM II

339.1 ELECTRON MICROSCOPIC DEOXYGLUCOSE AUTORADIOGRAPHY OF "SLAM" FROZEN OLFACTORY BULB. T. E. Benson,[1] P. E. Pedersen,[2] G. D. Burd,[3] D. M. D. Landis[3] and G. M. Shepherd.[1] [1]Sect. Neuroanatomy, Yale U. Sch. Med., New Haven, CT 06510. [2]Rockefeller University, New York, NY 10021. [3]Dept. Neurology, Massachusetts General Hospital, Boston, MA 02114.

We have employed the quick freezing technique of Heuser, Reese and Landis (Cold Spr. Harb. Symp. Quant. Biol., 40: 17, '75) in the development of a method for 2-deoxy [^{3}H] glucose (2DG) electron microscopic (EM) autoradiography (ARG).

Twelve day rat pups were injected intraperitoneally with 150 µCi/g 2DG and suckled their dams for 60 minutes. Coronal slabs of freshly dissected olfactory bulb were frozen against a liquid He chilled copper plate and the frozen tissue simultaneously acetone substituted and osmicated. Substituted tissue was embedded in plastic/silicone. One or 2 µm sections were cut on a dry knife and spread on anhydrous glycerol for light microscopic (LM) ARG. Eighty nm sections were cut onto glycerol for EM ARG. Photographic emulsion (Kodak NTB-2 for LM, Ilford L4 for EM) was placed over carbon-coated sections with an expanding loop device (Telford & Matsumura, Stain Technol., 44: 259, '69).

"Slam" freezing yielded ultrastructural preservation of all bulbar laminae. Scintillation counts from fluids used in the processing of "slam" frozen tissue indicated that tracer washout was negligible. In addition, 2DG-6-phosphate did not diffuse into the glycerol used for sectioning.

EM autoradiographs corroborated these findings. It was also apparent that tracer translocation did not occur during emulsion application. Observations of the tissue/plastic interface revealed high grain densities over bulbar tissue, but only background or "crossfire" grains over the plastic.

LM autoradiographs showed patterns of label uptake similar to those observed with more conventional freezing (Lancet et al., Proc. Natl. Acad. Sci. USA 79: 670, '82). In our preliminary observations of EM autoradiographs, silver grains were seen over terminals and dendritic cytoplasm in the neuropil of the external plexiform layer of the olfactory bulb.

Because of the apparent absence of tracer washout or translocation, this method appears to be suitable for the analysis of the incorporation of 2DG (or, perhaps, other diffusable substances) at the level of cellular processes and cell organelles. Technical aspects of this method which extends the 2DG neurofunctional mapping technique to the ultrastructural level will be discussed.

Supported, in part by USPHS NRSA 5 F 32 NS06990-02 and NS 16993.

339.2 REGIONAL DISTRIBUTION OF CHANGES IN LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU) IN THE RAT BRAIN INDUCED BY A DOPAMINE ANTAGONIST (HALOPERIDOL) AND AGONIST (BROMOCRIPTINE). G. Pizzolato,* T.T. Soncrant,* and S.I. Rapoport (SPON: N.R. Cutler). Lab of Neurosciences, Gerontology Res. Ctr., Natl. Inst. on Aging, NIH, Balto. City Hospitals, Baltimore, Maryland 21224.

Pharmacological effects of haloperidol (HAL) and bromocriptine (BRO) are thought to be mediated by their action on dopamine (DA) receptors. Distinct sub-populations of DA receptors in the brain are known to be differently influenced by these two drugs. We used the functional mapping technique of Sokoloff et al. (J. Neurochem. 28:897, 1977) to determine if the distribution of altered regional functional activity in response to these drugs correlates with the known anatomy of the DA system or with the distribution of DA receptors.

Femoral artery and vein catheters were inserted in 3 mo old, male Fischer-344 rats under ether anesthesia, 5.5 h before i.v. injection of [14-C]-deoxyglucose (DG). Animals received HAL 1 mg/kg i.p. 1 hr before DG, BRO 20 mg/kg i.p. 1,2,3, or 4 h before DG, or vehicle. LCGU was determined in 59 anatomic regions.

HAL significantly depressed LCGU (average 23%) in 26 of the regions examined and caused an increase only in the lateral habenula. Many of the areas affected are known to be rich in DA terminals or to be innervated by dopaminergic neurons (anterior medial and prefrontal cortex, dorsal hippocampus, lateral habenula, subthalamic nucleus, bed nucleus of stria terminalis, diagonal band nucleus, locus coeruleus, cerebellum). However, LCGU was not altered in other dopaminergic regions (substantia nigra, ventral tegmental area, arcuate nucleus, striatum, accumbens, anygdala, lateral septum, olfactory tubercle). HAL also reduced LCGU in regions not known to contain DA terminals (neocortex, thalamus, medial geniculate, superior colliculus).

BRO reduced LCGU in about 20% of the regions after 2 h (anterior medial and visual cortex, dorsal hippocampus, striatum, accumbens, ventral tegmental area, substantia nigra compacta, arcuate, lateral geniculate, lateral preoptic area, locus coeruleus, superior olive). The reduction persisted in many cases at 4 h. At 4 h BRO significantly increased LCGU in other regions (somatosensory cortex, VPM thalamus, nucleus of spinal tract of V, posterior vermis, zona incerta).

These findings show a more widespread functional involvement of the brain in response to either BRO or HAL than expected on the basis of the known topography of the dopaminergic system. The existence of many functionally important multi-synaptic circuits is suggested by the results. The different patterns of local metabolic activity produced by HAL and BRO may be due to their different actions on discrete sub-populations of DA receptors.

339.3 EFFECT OF AN ANESTHETIC DOSE OF PHENOBARBITAL (PHE) ON LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU) IN RAT CEREBRAL CORTEX. J.E. Hodes*, T.T. Soncrant* and S.I. Rapoport. Laboratory of Neurosciences, Gerontology Research Center, Natl. Inst. on Aging, Balto. City Hospitals, Baltimore Maryland 21224.

Barbiturates produce dose-dependent depression of CNS function from mild sedation to general anesthesia. PHE has a selective anticonvulsive effect at doses that cause minor, if any, depression of the reticular activating system. Larger doses cause profound decreases in level of conciousness accompanied by changes in E.E.G., respiratory depression, myocardial depression, and decreased cerebral oxygen consumption. We used the (14-C)-2-deoxyglucose (2-DG) technique of Sokoloff et al. (J. Neurochem. 28: 877, 1977) to measure LCGU in cortical regions of the rat brain to see if an anesthetic dose of PHE decreases the functional activity of the brain.

Femoral artery and vein catheters were placed in 3 mo old, Fischer-344 rats under light halothane anesthesia, 5.5 prior to i.v. injection of 2-DG. Animals received sodium PHE 180 mg/kg or saline i.p. 1 h before 2-DG, and mean blood pressure (BP) and heart rate (HR) were periodically monitored. LCGU was determined in 27 cortical regions. In a separate experiment arterial pO2, pCO2, and pH were measured before and 1 h after PHE 180 mg/kg i.p.

BP and HR were significantly reduced in PHE-treated rats (89 ± 9 mm Hg, 312 ± 34 bpm) when compared to controls (125 ± 4 mm Hg, 445 ± 17 bpm). Arterial pH, pO2, and pCO2, were unaffected. LCGU declined significantly after PHE in all cortical areas examined. Heterogeneity in LCGU among cortical layers was reduced. LCGU in layer IV of some areas are shown below (*p <.05).

REGION	LCGU (μmoles/100 g/min) (Mean ± SEM) Control (n=4)	PHE 180 mg/kg (n=3)
Pre-frontal Ctx	95 ± 7	57 ± 6*
Frontal Ctx	100 ± 9	58 ± 6*
Motor Ctx	99 ± 9	54 ± 8*
Auditory Ctx	132 ± 10	69 ± 11*
Visual Ctx	92 ± 4	60 ± 10*

These data show widespread depression of functional activity in cortical areas of the brain in response to an anesthetic dose of PHE. This profound and generalized decline in LCGU is similar to that seen during anesthesia induced by other agents (Sokoloff et al., Grome and McCulloch J. Neurochem. 40: 569, 1983). Whether the decrease in LCGU after PHE is primarily due to a direct effect on cortical function or is produced by alterations in cardiovascular status remains to be determined.

339.4 EFFECTS OF CAFFEINE ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN RAT. A. Nehlig*, G. Lucignani*, M. Kadekaro, L. Porrino, & L. Sokoloff. (SPON:C. Kennedy). Laboratory of Cerebral Metabolism, NIMH, Bethesda, MD 20205.

Caffeine is a widely used stimulant, ingested daily by most people in coffee, tea, and soft drinks. Behaviorally, it induces alertness and enhances locomotor activity. It is also known to exert a diuretic effect. Caffeine's main pharmacological effects are exerted on the central nervous system, cardiovascular, and renal systems. It is known to be an inhibitor of phosphodiesterase, and, therefore increases the amounts of cAMP in brain. In an attempt to localize the actions of caffeine within the brain local cerebral glucose utilization was studied with the quantitative autoradiographic 2-[^{14}C]deoxyglucose method of Sokoloff et al. (J.Neurochem. 28, 897-916, 1977) in 4 groups of rats administered normal saline, 0.1, 1.0 or 10 mg/kg caffeine. Caffeine or saline was injected intravenously 15 min before initiation of the 2-DG procedure. After caffeine injection, blood pressure of all the animals remained almost unchanged, and arterial blood gases and pH were not significantly affected except at the highest dose (10 mg/kg) at which there was a statistically significant decrease in arterial pCO_2 and increase in arterial pO_2. Striking behavioral changes were noticed only at the highest dose (10 mg/kg); these included intense grooming, sniffing, and head bobbing. At the lowest dose (0.1 mg/kg), increases in glucose utilization were restricted to the trigeminal motor nucleus involved in chewing behavior and to the medial habenula. Most effects of caffeine were seen only at the highest dose (10 mg/kg). At that level, increases in glucose utilization occurred in areas involved in motor control, mainly the extrapyramidal dopaminergic pathway, e.g., substantia nigra, caudate, globus pallidus, entopeduncular and subthalamic nuclei. Also significantly affected were the ventral tegmental area, anterior cingulate cortex, medial prefrontal cortex, septum and raphe nuclei. The highest dose also increased glucose utilization in structures involved in the regulation of endocrine function, e.g., paraventricular nucleus, arcuate nucleus, and the median eminence. The behavioral effects of caffeine are, therefore, well-correlated with the stimulation of metabolic activity in a variety of cerebral structures related to the control of motor activity and alertness.

339.5 PITUITARY GLAND DOES NOT RELY HEAVILY ON GLUCOSE FOR ENERGY METABOLISM. J.R. Viña*, R. Page*, D. Davis* and R. Hawkins. Departments of Anesthesia, Physiology and Surgery, Hershey Medical Center, The Pennsylvania State University College of Medicine, Hershey, PA 17033.

Previous results from this laboratory showed conclusively that glucose was not an important fuel of oxidative metabolism in vivo in the neurohypophysis, adenohypophysis or the pineal gland of rats (1). Each of these structures lacks a blood-brain barrier. No oxidation of glucose by the Krebs cycle could be detected in 48-hour fasted rats and very little oxidation occurred in fed rats. On the other hand, fatty acids were oxidized to an appreciable extent. This metabolic pattern contrasted with other cerebral structures (which have a blood-brain barrier) in which glucose was the prime fuel (eg. cortex and striatum). Since more than 90% of the energy from glucose is generated by the Krebs cycle, it is clear that little energy is available from glucose in the pituitary or pineal glands. On the other hand there is evidence that net uptake of glucose occurs. Label from ^{14}C-deoxyglucose is accumulated by the pituitary, presumably as ^{14}C-deoxyglucose phosphate (2,3). This suggests that net phosphorylation of glucose does occur in vivo. If glucose is taken up but not oxidized then what is its fate? There are two possibilities; oxidation by the hexose monophosphate shunt, or conversion to lactate. In order to determine the metabolic fate of glucose we measured arteriovenous differences across the pituitary of pentobarbital-anesthetized female pigs. Pigs were chosen because the pituitary can be surgically exposed. Also the pituitary of pigs has an accessible vein for blood sampling. The arteriovenous difference of glucose was 0.53 ± 0.27 (SE) μmol/ml and the venoarterial differences of lactate and pyruvate were 1.0 ± 0.26 (SE) μmol/ml and 0.06 ± 0.02 (SE) μmol/ml respectively. These results show that all the glucose taken up was metabolized by glycolysis and converted primarily to lactate. From this we can infer that the pyruvate dehydrogenase complex is inactive. If the hexose monophosphate shunt pathway was active, it must have consumed only a very small amount of glucose. Taken together with earlier results it can be concluded that glucose is not an important energy source in the pituitary. Furthermore, the results suggest that deoxyglucose is not suitable for the measurement of energy metabolism in the pituitary; instead, all the metabolic fuels of respiration will need to be measured.

Supported in part by NIH grants NS 16737 and NS 15926.

1) Vannucci et al., Trans. Am. Soc. Neurochem. 13(1982)201
2) Schwartz et al., Science 205(1979)723-725
3) Gross et al., Abstracts Soc. Neuroscience 8(1982)85

339.6 LOCAL CEREBRAL DISTRIBUTION AND METABOLISM OF 1-14C-OCTANOATE - A FAST FUNCTIONAL MARKER, R. C. Collins, E. Santori*, and T. Der. Dept. Neurology, Wash. Univ., St. Louis, MO 63110

Octanoate is an eight carbon fatty acid that exists in trace concentrations in tissues as an intermediary metabolite. When injected intravascularly in rats it readily crosses the blood brain barrier with uptake equal to 94% compared to water (Oldendorf, 1973). It is metabolized by β-oxidation in mitochondria with the C-1 carbon becoming primarily localized in glutamate and glutamine over one to five minutes, or lost as CO_2 (Cremer, 1977).

We have studied local cerebral octanoate distribution and metabolism in 300 g albino rats using 1-14C-Octanoate (Octo; 10μC/100gm iv; SA=30 mCi/mmol) and the film autoradiographic technique. Isotope was injected in 50μl of saline in 1 sec through a catheter in the superior jugular vein in awake animals. Decapitation was performed between 10 seconds and 30 minutes, the brain frozen and cut at 20μm for one week exposure to XAR film.

OCTO autoradiograms of animals killed between 20 seconds and 2 minutes showed a degree of anatomical resolution similar to 14-C-deoxygucose (DG) autoradiography. Final tissue concentrations of isotope were measured densitometrically (mμci/gm) for 15 grey or white matter structures and compared to control rates of DG metabolism (μm/g/min). There was a high correlation for OCTO animals killed between 20 seconds (r=.96) and 2 minutes (r=.89), but not beyond (6 min, r=.67; 30 min, r=.39). In separate studies the distribution of mitochondria was measured using diaminobenzidine (DAB) histochemistry. There was no correlation between local OCTO and local DAB (OD at 490; r=.5).

Changes in "functional metabolism" were measured by visual stimulation of unilaterally enucleated rats. Eye removal resulted in a 20 to 30% reduction of OCTO labeling in contralateral LGNd and superior colliculus (SC) in ambient light. Photic flash stimulation between 4 and 16/sec increased OCTO in innervated LGN and SC between 10 and 80%. This increment in labeling was at its peak at 20 seconds following iv injection and disappeared beyond 2 minutes.

These studies suggest free arterial OCTO is cleared rapidly by brain like non-polar markers of blood flow. The high autoradiographic resolution and 2 minute stability in control and functional studies suggest transient "trapping" of some label by rapid metabolism. The loss of functional labelling and decay of images beyond two minutes of study suggest that subsequent metabolism of C-1 OCTO is largely unrelated to brain energy needs.

W.H. Oldendorf, Amer. J. Physiol. 224:1450-1453, 1973
J.E. Cremer, et. al., J. Neurochem. 28:215-222, 1977

339.7 PET IN SCHIZOPHRENIA AND AFFECTIVE ILLNESS
M.S. Buchsbaum,* J.C. Wu,* H.H. Holcomb, L.E. Delisi,* E. Hazlett,* R. Ball* (SPON: E. Usdin) Department of Psychiatry, University of California, Irvine; Lab. Psych. NIMH

Local cerebral uptake of deoxyglucose labeled with fluorine (FDG) was measured by PET. The subject groups consisted of 16 patients with schizophrenia (11 m, 5 w, 28.3 ± 7.7 yrs), 11 patients with affective illness (7 m, 4 w, 41.7 ± 9.9 yrs.) and 19 normals (13 m, 6 w, 31.1 ± 10.5 yrs). Patients were diagnosed using DSM-III criteria and maintained off medication a minimum of 14 days (average 39.8 days) by the clinical units of NIMH (Drs. Post, Cohen, Morihisa,Weinberger, Pickar) and MD State Research Institute (Dr. Carpenter).

Subjects received 3-5 mCi of FDG while receiving unpleasant electrical stimulation to their right forearm which occured 1/sec for 34 mins. Shocks were of 4 intensities (2,9,16,23 mAm) and were given in random order. Six sets of 256 stimuli were presented for a total of 1500 brief shocks. Subjects were given FDG with their eyes closed in a darkened and acoustically attenutated chamber. Subjects were monitored to insure that eyes were closed. After 32-38 minutes of stimulation in a controlled environment, subjects were transferred to the Ortec Ecat II scanner where 7 to 8 scans parallel to the canthomeatal line were completed. Raw counts were converted to glucose (micromoles/100 gm/min) using the Sokoloff three-constant model. For each brain slice, the outer brain cortex was automatically outlined. A 2.2 cm thick cortical peel was digitally defined and placed in a scaled height proportional to distance from the CM line in a lateral equal area brain map. A lateral topographic view was reconstructed by interpolation between the slices.

Normals and patients both showed an anteroposterior gradient. Data was analyzed by normalizing all pixels within the map and calculating t-tests between groups on each pixel. Maps of the t-test revealed normal/schizophrenic differences most marked in the superior frontal lobe (normals higher) and the posterior occipital region (schizophrenics higher) and normal/affective differences most marked in frontal and parietal lobes (normals higher) and posterior occipital (affectives higher). Maps of the variances of glucose use showed the greatest variance in the somatosensory area of the schizophrenics indicating great individual differences in stimulus response. Lack of activation of these areas is a possible interpretation. Variance maps in the normals and affectives were radically different from the schizophrenics and showed a peak in the temporal regions. Taken together, these findings are consistent with abnormal sensory regulation in schizophrenia.

339.8 THREE-DIMENSIONAL RECONSTRUCTION OF BRAIN METABOLISM.
L.S. Hibbard* and R.A. Hawkins, (SPON: W. Severs), Departments of Anesthesia and Physiology, Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033

We are using the three-dimensional reconstruction of autoradiographs, made from serial brain sections, to study a variety of neurochemical problems. Our method overcomes some of the limitations of conventional analyses. Currently, film optical densities are measured manually at locations corresponding to anatomic structures which are identified by the experimenter. This limits the amount of data which can be collected and the objectivity of its measurement. Using a computer, all the serial images from an experiment are digitized at high resolution, the optical densities are converted to metabolic rates, and the data are stored for subsequent processing.

The reconstruction of digitized images is carried out by a system of computer programs which we developed. Reconstruction involves several tasks: the alignment of single coronal images, the calculation of images of the means from several individual experiments (and their standard deviations), the calculation of the images of the difference between two data sets, and the calculation of maps of the bilateral asymmetry of the brain.

Image alignment is effected by application of the principal axis transformation from classical mechanics. The image is taken to be a rigid body. The principal axes, calculated for the point at the center of mass of the image, determine the amounts of rotation and translation required to place the image at an established reference position. Thus transformed, each image is aligned with the others in the set.

Alignment enables the comparison of different sets of serial images. Identically prepared data sets are combined to form mean and standard deviation serial images. Homologous images from the different sets are compared by subtracting one from the other to form images of the differences. Statistically significant differences can be readily identified by constructing images of differences estimated to be significant within specified confidence limits. The extent of bilateral asymmetry can be mapped by subtracting one half of an aligned data set from the other and displaying the differences.

Computer reconstruction expands the scope of analyses of brain metabolism in several directions: toward greater detail at the structural level, toward a global view of the brain as a composite of many parts, and toward more reliable measurements of metabolic and functional changes.

Supported in part by NIH grant NS 16739 and a grant from The Whitaker Foundation.

339.9 ^{31}P NMR SPECTROSCOPY OF HIGH ENERGY PHOSPHATE COMPOUNDS IN THE RAT BRAIN WITH CHRONIC SURFACE COILS.
A.L. Benabid, M. Decorps*, J.F. Lebas*, J.L. Leviel*. LMCEC-CERMO, Université de Grenoble, and ORF-LM, Centre d'Etudes Nucléaires, 38041, Grenoble Cedex, France.

Nuclear magnetic resonance (NMR) spectroscopy in vivo provides a powerful tool for biochemical studies of organs in situ. Chronically implanted radiofrequency surface coils (SC) were developped for long lasting experiments. SC were made of two turns of copper wire solenoïds, 20 mm in diameter, connected to male pins parrallel to coil axis. Adult rats were anesthetized, placed on a stereotactic frame, the skull was exposed , four teflon screws were implanted into the parietal bones. The SC was positionned horizontally over the skull. The SC and the screws were embedded with methyl metacrylate. Animals were allowed to recover from surgery for a week. A home-built NMR probe was designed wich could fit the wide bore (87 mm diam.) of a 4.7 Teslas supermagnet of a CXP-200 Bruker spectrometer. This probe has female connectors, where the implanted SC could be plugged in, and magnetic capacitors used for tuning the probe to the proper Larmor frequency of the investigated nucleus. At time of the experiment, the unanesthetized animal is placed into the probe, its SC is plugged into the female connectors, and the probe is introduced into the magnet. Field homogeneity is shimmed using the proton resonance signal. With the same coil, spectra of ^{1}H, ^{23}Na, ^{31}P were observed within 150 seconds. ^{31}P spectra exhibit peaks corresponding to sugar phosphates (SP), inorganic phosphate (Pi), phospho-diesters (PDE) and high energy compounds as phosphocreatinine (PCr) adenosine di (ADP) and triphosphate (ATP). The chemical shift of the Pi peak provides measurement for tissues pH. Effects of hypoxia, acute sublethal intoxication by potassium cyanide (KCN) (5 mg/kg ip) and status epilepticus induced by bicuculine (12 mg/ kg ip) on brain metabolism were studied using 31 P - spectroscopy in vivo. Within 5 mn after KCN injection, PCr and pH decrease, Pi increases, while ATP and ADP are not altered. These parameters return to normal values within one hour. Intensity and duration of these metabolic changes are dose dependent. Hydroxocobalamine reverses or prevents these changes depending on its time of administration. This study provides a new insight into the mitochondrial mechanisms of oxidative phosphorylation in vivo, the enzymatic parameters of which could be investigated using more sophisticated NMR methods, as saturation transfer and 13 C. NMR spectroscopy in vivo.

339.10 EVIDENCE FOR THE CEREBRAL UPTAKE IN VIVO FROM TWO POOLS OF GLUCOSE AND THE ROLE OF GLUCOSE-6-PHOSPHATASE IN REMOVING EXCESS SUBSTRATE FROM BRAIN.
W. Sacks, S. Sacks* and A. Fleischer*. New York State Rockland Research Institute, Orangeburg, NY 10962.

In our arterio-venous (A-V) method for the determination of cerebral metabolism in humans in vivo (Handbook of Neurochem., A. Lajtha, ed., Plenum, New York, 1983, pp. 321-351) we frequently observed a lack of agreement of the cerebral uptake of ^{14}C-glucose with 'cold' glucose. This led to our use of ^{14}C-glucose-6-phosphate in our study showing the conversion of that compound to ^{14}C-glucose by human brain in vivo. In our single injection experiments, the extraction (A-V/A) of ^{14}C-glucose was often less than that of 'cold' glucose especially with rapidly falling arterial blood radioactivity. In contrast, in our constant infusion experiments, with rapidly rising arterial radioactivity, the extraction of ^{14}C-glucose exceeded that of unlabeled glucose. These observations led us to hypothesize that glucose-6-phosphatase in brain functions to remove excess substrate (in the form of glucose-6-phosphate) from brain by dephosphorylation. However, in those constant infusion studies in which arterial ^{14}C-glucose radioactivity increased slowly, the extraction of ^{14}C-glucose varied considerably. At times, within the same experiment, it greatly exceeded that of 'cold' glucose while in other A-V samples it was considerably less than that of unlabeled glucose. The data resembled closely those of our studies of the cerebral metabolism of glucose anomers in human subjects in vivo (Brain Metabolism and Cerebral Disorders, H.E. Himwich, ed., Spectrum, Flushing, NY, 1976, pp. 89-127). That the extraction of ^{14}C-glucose could be greater than that of 'cold' glucose with slowly rising arterial radioactivity could be explained by assuming a slight delay (about 20 sec) in the conversion of ^{14}C-glucose-6-phosphate to ^{14}C-glucose (by glucose-6-phosphatase) before leaving the brain via the circulation. That the cerebral uptake of ^{14}C-glucose could be significantly less than that of 'cold' glucose led to the hypothesis that the brain must select from two pools of glucose. These would most likely be α-glucose and β-glucose and they would probably be utilized at different rates by both the body and the brain. The presence of significant amounts of mutarotase in brain and other body organs makes such an hypothesis feasible. Work is currently underway in our laboratory on a method to separate and assay radioactivity of glucose anomers in whole blood to obtain further evidence for our hypothesis.

339.11 EVIDENCE FOR THE PRESENCE OF TWO FORMS OF GDH IN MAMMALIAN TISSUES: ONE IS REDUCED IN A NEUROLOGICAL DISORDER A. Plaitakis*, A. Colon*, A. Perrakis*, S. Berl and D.D. Clarke*,#. Dept. of Neurology, Mount Sinai School of Medicine, New York, N.Y. 10029, #Dept. of Chem., Fordham Univ., Bronx, N.Y.

A partial deficiency of glutamate dehydrogenase (GDH) and abnormal glutamate metabolism have been described in patients with an adult onset neurological disorder with mutliple system atrophy (Plaitakis et al., Science 216:193, 1982). To further characterize this enzymatic defect, whole leukocyte homogenates, prepared after cell disruption by freeze-thaw, were fractionated by differential centrifugation into 100,000xg "soluble" and "particulate" components. These fractions were tested for heat stability at various temperatures, pHs and buffer ionic strengths. At 45°C in 50 mM Tris HCl, pH 7.40 buffer, the half life of the "soluble" fraction was approximately 10 hours whereas that of the "particulate" fraction was 40 minutes with similar protein concentrations. Increasing the pH to 8.00 and 8.50 increased the rate of heat inactivation. The "particulate" fraction had a half life of approximately 13 and 4 minutes, respectively, whereas the "soluble" enzyme's half life was 4 hours and 1 hour, respectively. At 47.5°C in 50 mM Tris-HCl, pH 7.40 ,the half life of the "soluble" fraction was approximately 5 hours and of the "particulate" fraction 24 minutes. The presence of 10 mM L-leucine or 154 mM sodium chloride in the incubation media protected both fractions against heat inactivation. Preliminary studies with rat brain homogenates showed that GDH could be similarly fractionated into "soluble" and "particulate" components which differ in thermal stability. The "heat-labile" and "heat-stabile" forms of GDH were determined in whole leucocyte homogenates from patients with a partial deficiency of the total GDH activity and controls by measuring loss of activity after incubation at 45°C in 50 mM Tris HCl, pH 8.00 (or 47.5°C, pH 7.4) for 60 minutes. There was an almost complete deficiency of the "heat-labile" GDH fraction in the patients whereas the "heat-stable" fraction was the same in both groups. These results support the existence of two GDH "isoenzymes" in mammalian tissues, one of which is deficient in these neurologic patients.

Supported in part by NIH grants NS-16871 and NS-11631, The Clinical Center for Research in Parkinson's and Allied Diseases.

339.12 A MATRIX METHOD TO QUANTITATE FUNCTIONAL INTERACTIONS AMONG BRAIN REGIONS: APPLICATION TO STATE OF REDUCED SENSORY INPUT. R. Duara,* B. Horwitz, C.L. Grady,* N.R. Cutler, and S.I. Rapoport. Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, Bethesda, MD 20205

It has previously been demonstrated in animals by Sokoloff that regional glucose metabolism (rCMRglc), measured by means of [14C]-2-deoxyglucose (2DG), correlates with regional neural activity. This technique has been applied to humans using [18F]fluoro-2DG (FDG) and positron emission tomography (PET). Thus, the Sokoloff technique permits one to obtain indirectly a map of neural activity for the entire brain.

The glucose utilization rates for each brain region for a specified and controlled environment show considerable variability, due partly to experimental measuring errors, and partly to variability intrinsic to the brain of the subject. By focusing on the latter, we can determine which regions of the brain are functionally coupled under the stimulus environment. We introduce a matrix whose elements are correlation coefficients obtained by correlating rCMRglc values between all pairs of defined brain regions, for all subjects studied. The fundamental hypothesis made is that the larger the correlation coefficient, the stronger the functional interaction between the two regions. Thus, this method gives us a quantitative measure of the functional relationships in a specified brain state for a defined subject population.

To illustrate this procedure, we examined during reduced visual and auditory input 40 healthy adult men whose rCMRglc values were obtained with FDG and PET. The strongest correlations were between bilaterally symmetric homologous brain regions, and represent left-right symmetry seen on the individual PET scans. We also found strong correlations between many regions in the frontal and parietal lobes, between regions in the temporal and occipital lobes, and few correlations between the frontal-parietal and temporal-occipital domains. This finding, although not unexpected in a state where there is little need for the processing of visual and auditory information, is not directly manifest from the individual scans, and thus represents a new way to characterize more fully the brain state under study.

339.13 DIFFERENT PATTERNS OF LOCAL CEREBRAL GLUCOSE UTILIZATION: SELF STIMULATION VS. EXPERIMENTER-IMPOSED STIMULATION TO THE INTRACRANIAL VENTRAL TEGMENTAL AREA. L.J. Porrino, R.U. Esposito, T. Seeger*, A.M. Crane*, A. Pert, NIMH, Bethesda, MD 20205.

The quantitative 2-[^{14}C]deoxyglucose technique (J.Neurochem. 28, 897-916, 1977) was used to measure local cerebral glucose utilization (LCGU) in freely moving rats working for rewarding brain stimulation to the ventral tegmental area (VTA). In order to isolate areas functionally involved in the performance of this goal-oriented task 3 groups of rats were tested: 1) rats lever-pressing to receive intracranial electrical stimulation to the VTA contingently (ICSS); 2) rats receiving electrical stimulation to the VTA noncontingently, delivered by the experimenter (NCON); and 3) animals implanted with electrodes in the VTA but not electrically stimulated (UNSTIM). Male Sprague-Dawley rats were implanted with bipolar platinum electrodes and screened for self-stimulation to the VTA (parameters: biphasic rectangular wave pulses; 100 Hz; 250-300μA; 400 msec trains; 60-80 responses/min). All rats were trained for ICSS, but those in the NCON and UNSTIM groups were then extinguished. Experimental sessions were initiated when the rat was placed in the chamber and stimulation begun: ICSS-lever pressing; NCON-experimenter imposed stimulation at its preferred rate; UNSTIM-no stimulation. 2-DG was injected 10 minutes later, and the standard protocol for determination of LCGU was followed. LCGU was markedly increased in the NCON group in a discrete area around the electrode tip and in projection fibers extending rostrally in the lateral medial forebrain bundle into the diagonal band of Broca. The pathway could be followed caudally into the pontine reticular formation. Ipsilateral to the stimulation site, increased LCGU was seen throughout the limbic and cortical terminal fields of the VTA. The subset of structures activated in each NCON rat was dependent in part on electrode placement. Bilateral increases in LCGU relative to UNSTIM rats were evident in thalamic and cortical sensory-motor areas reflecting behavioral activation. In the ICSS group the pattern of metabolic activity at the electrode site and in pathways was similar to the NCON, but quite different in VTA terminal fields. Rostrally, ipsilateral increases in LCGU were found consistently only in the central and basolateral amygdala, the medial prefrontal cortex, and bed nucleus of the stria terminalis. Caudally, the locus coeruleus and parabrachial nucleus were activated unilaterally. Bilateral increases in the lateral septum and hippocampus were noted. The 2-DG technique has allowed visualization of the underlying neuronal circuitry involved in the performance of a task for rewarding brain stimulation. Further, it has permitted a distinction between this circuitry and the pattern of activation resulting from non-contingent electrical stimulation.

340.1 EFFECTS OF MESOLIMBIC DOPAMINE DEPLETION ON A TEST OF ATTENTIONAL SWITCHING IN THE RAT. T.W. Robbins*, J.L. Evenden*, S. Wood* and P. Reading* (SPON: European Neuroscience Association). Dept. of Experimental Psychology, Univ. of Cambridge, Cambridge CB2 3EB UK.

Kristofferson's (1967) paradigm, which has been used for measuring attentional switching in normal and schizophrenic human subjects, has been modified to test the effects of d-amphetamine and 6-hydroxydopamine (6-OHDA)-induced lesions of the nucleus accumbens (N.Acc.) in the rat. The modified paradigm involved a discrimination of temporal order between two visual stimuli in a chamber (20 x 8 x 10 cm) with three response holes at one end monitored by infra-red beams. The 2 side holes could be illuminated by green lights provided by light-emitting diodes. During a trial, the two side-lights were turned on and programmed to offset after a variable interval (VI) at different delays. Sucrose solution was provided in the central hole if the rats indicated which of the two lights offset first by responding in the corresponding holes. Incorrect responses resulted in time-out (1 sec). After preliminary training of 12, food-deprived rats, sessions of 160 trials consisted of 20 different types of randomly intermixed trials with 5 different VIs (2½, 3, 3½, 4, 4½ sec) and 4 different delays (10, 50, 250, and 1250 msec). When better than 70% correct performance (at 250 msec delay) had been attained, 6 rats received stereotactic, bilateral injections of 6-OHDA(8µg base in 2µl vehicle over 5 min) through a 30 ga stainless steel cannula into the N.Acc. The other 6 rats received vehicle (0.2% ascorbate in 0.9% saline) as a sham control procedure. All rats were pretreated with 50 mg/kg pargyline. This 6-OHDA treatment was subsequently found to have depleted dopamine and noradrenaline by over 80% in N.Acc, but by only about 30% in corpus striatum. For the 10 day period immediately following surgery, the N.Acc. group were significantly less accurate and slower to make correct responses than the controls, at all delays. By 12-16 days post-operation, however, the performance of both groups was similar. Beginning about 28 days after surgery, doses of d-amphetamine (0.4, 0.8, 1.6, 2.3 mg/kg) were administered i.p. over the next 12 days in an ascending order to all rats. In the sham control group, accuracy of performance was significantly worsened at 1.6 and 2.3 mg/kg at all delays except 10 msec. Latencies to make the correct response were also significantly lengthened by these doses at all delays. These disruptive effects of d-amphetamine were significantly attenuated in the N.Acc group. The results suggest that 6-OHDA lesions of N.Acc., in the short term, and d-amphetamine, impair attentional switching, but that the disruptive effects of the drug are largely abolished by prior depletion of catecholamines from N.Acc.

Kristofferson, A. in Attention and Performance (A. Sanders Ed.) North Holland, Amsterdam, 1967, pp. 93-100

Supported by MRC grant 6979/1150/N to TWR and B.J. Everitt

340.2 HALOPERIDOL, BUT NOT CLOZAPINE, BLOCKED APOMORPHINE-INDUCED DISRUPTION OF SELECTIVE ATTENTION; WHEREAS CLOZAPINE, BUT NOT HALOPERIDOL, INTERFERED WITH MAINTENANCE OF ATTENTION IN GERBILS. MaryLou Cheal. Neuropsychology Laboratory, Ralph Lowell Laboratories, Mailman Research Center, McLean Hospital, and Department of Psychiatry, Harvard Medical School, Belmont, MA 02178*.

Using the stimulus-elicited investigation paradigm with Mongolian gerbils, the neuroleptics, haloperidol and clozapine, were compared for their effects on attentional behavior and for their ability to block apomorphine-induced disruption of selective attention. Haloperidol (0, .1, .3, or 1 mg/kg), the typical butyrophenone neuroleptic, decreased investigation of novel objects by gerbils following systemic injections. When given prior to apomorphine (1 or 3 mg/kg) haloperidol blocked apomorphine-induced disruption of selective attention in a dose dependent manner. Thus, haloperidol acts like pimozide in this paradigm (Cheal, Psychopharmacology, 1980, 69, 93-100). In contrast, clozapine (0, .03, .1, .3, 1, or 3 mg/kg), the atypical dibenzodiazepine neuroleptic, increased frequency of investigation both following injection and 24 hours later, suggesting interference with maintenance of attention similar to that shown with the acetylcholine antagonist, scopolamine (Cheal, Behavioral and Neural Biology, 1981, 33, 163-187). When given with apomorphine (1 mg/kg), clozapine did not block apomorphine effects on selective attention. The results are discussed in relation to differential effects of these neuroleptics on other behaviors, and to their individual pharmacological profiles.

*Present address: Laboratory Animal Care Program, Arizona State University, Tempe, Arizona 85287. This research was partially supported by BRSG Grant RR05484 awarded by the Biomedical Research Support Program, DRR, NIH, to McLean Hospital.

340.3 STRIATAL DOPAMINE DEPLETION ATTENUATES ANOREXIA PRODUCED BY D-AMPHETAMINE. E.M. Joyce*and S.D. Iversen*. (SPON: Europ. Neurosci. Assoc.). Dept. of Expt. Psychol., Univ. of Cambridge, U.K.

Psychomotor stimulant drugs such as amphetamine are also commonly used anorectic agents, but the relationship between the stimulant and anorectic responses remains obscure. Previous evidence has suggested that the locomotor stimulant effect of amphetamine in rats can be abolished by 6-hydroxydopamine (6-OHDA)-induced depletion of dopamine (DA) from the nucleus accumbens without attenuating the anorectic response. In the present experiment we show, in contrast, that DA depletion from the caudate/putamen, which reduces the stereotyped response to amphetamine, also attenuates amphetamine anorexia. Male albino rats were given 50mg/kg of pargyline followed by bilateral injections of either 6-OHDA (8ug in 2ul vehicle over 2m) or its vehicle (0.2% ascorbate in 0.9% saline) into the head of caudate nucleus (A + 2.0 from bregma, L ±3.5, V - 5.5 from dura).This treatment produced a significant depletion of DA only in caudate/putamen, but not in frontal cortex, nucleus accumbens or hypothalamus and there were no effects on noradrenaline concentration in frontal cortex or hypothalamus. At least 14 days after surgery locomotor activity of the lesion and sham groups was measured in photocell cages in response to d-amphetamine (0.5, 1.5 and 5.0mg/kg) or saline in a counterbalanced sequence with the rats 23 hours food deprived. Food was also available in the 30m test session. The lesioned group showed a significant dose-dependent increase in locomotor activity in response to amphetamine and also a dose-related significant attenuation of the anorectic response to the drug. These results 1) show that striatal DA depletion increases the locomotor response to d-amphetamine although the stereotyped response has been previously shown to be reduced; 2) confirm that stimulant-induced locomotor activity resulting from striatal DA depletion is not necessarily incompatible with eating and cannot in itself account for the anorectic response; 3) demonstrate that reduction in the anorectic response to intermediate doses of amphetamine can be produced by DA depletion of the corpus striatum and 4)show that although the anorectic response to amphetamine may consist of specific components at low doses it is exacerbated at high doses by the production of stereotyped responses incompatible with eating.

340.4 DISCRIMINATIVE STIMULUS CHARACTERISTICS OF ANTIDEPRESSANTS IN PIGEONS. T. Yamamoto* and J.H. Woods Dept. of Pharmacology, Univ. of Michigan Med. Sch., Ann Arbor, MI 48109.

Antidepressant drugs have been difficult to study using drug discrimination procedures in rodents (e.g., Howard, J.L., Soroko, F.E., and Cooper, B.R. in Enna, S.J. et al. (Ed.) Antidepressants Raven Press, New York, 1981, p. 115). We recently reported (Yamamoto, T., Solomon, R.E., and Woods, J.H. Pharmacologist, Fall Meeting Abstracts, 1983) that imipramine (3.2 mg/kg i.m.) produced significant drug-appropriate responding in pigeons trained to discriminate either quipazine (1.0 mg/kg i.m.) or d-amphetamine (1.8 mg/kg i.m.) from saline. The birds were trained under fixed-ratio schedules of food delivery to peck one key following drug administration and another key following saline administration. Since the drug discrimination procedure is similar to those used in rodents, this result with imipramine suggests the pigeon might be a useful drug discrimination preparation for the study of antidepressants. To explore this notion, a number of other antidepressants have been studied. The following compounds, when given intramuscularly, produce dose-dependent, substantial, though not necessarily complete, drug-appropriate responding in both groups of birds: desmethylimipramine (0.1-3.2 mg/kg), chlorimipramine (1-10 mg/kg), mianserin (3-18 mg/kg), pargyline (10-50 mg/kg) and iprindole (10-50 mg/kg). Nomifensine (0.1-5.6 mg/kg) produced substantial drug-appropriate responding in only the amphetamine-trained group. In contrast, fenfluramine (1-10 mg/kg) produced only quipazine-appropriate responding. Thus, drugs other than antidepressants are active in the preparation. In a three-key drug discrimination procedure in which a group of pigeons have been trained to discriminate quipazine from amphetamine from saline, we have found less cross generalization than with the two-key procedure between quipazine and amphetamine. In addition, imipramine produces only amphetamine-appropriate responding. It may be that the three-key procedure will provide a more specific characterization of the discriminative stimulus characteristics of antidepressants when compared to the two-key procedure. Research supported by USPHS Grant DA 00154 and a M.H. Seevers International Fellowship.

340.5 PIRENPERONE, A $5HT_2$ ANTAGONIST, ATTENUATES THE BEHAVIORAL EFFECTS OF LYSERGIC ACID DIETHYLAMIDE, 2,5-DIMETHOXY-4-METHYLAMPHETAMINE AND QUIPAZINE BUT NOT LISURIDE. David J. Mokler and Richard H. Rech. Dept. of Pharmacol./Toxicol. and Neuroscience Prog., Mich. State Univ., East Lansing, MI 48824.

Pirenperone (PIR) is one of a series of compounds which has a high, specific affinity for the H^3-spiriperidol-labelled 5-hydroxytryptamine receptor ($5HT_2$ receptor). Colpaert et al. (J. Pharmacol. Exp. Ther. 221, 206, 1982) reported that PIR (40 and 160 µg/kg) antagonized the discriminative stimulus cues of lysergic acid diethylamine (LSD) in rats. The purpose of the present experiment was to examine the effects of PIR on the disruption of operant behavior induced by LSD, 2,5-dimethoxy-4-methylamphetamine (DOM) and the non-hallucinogens quipazine (QUIP), a 5HT agonist, and lisuride (LIS), an analogue of LSD. Male Sprague-Dawley rats were food-deprived and trained to press a lever 40 times for one 45 mg food pellet (fixed ratio-40 [FR-40] schedule). Animals were administered 40 or 160 µg/kg PIR (40-min pretreatment) followed by LSD (25-400 µg/kg), DOM (0.125-4.0 mg/kg), QUIP (0.5-8.0 mg/kg) or LIS (5-160 µg/kg) (0-min pretreatment).

LSD, DOM, QUIP and LIS produced a dose-dependent decrease in reinforcements and increase in 10 sec periods of non-responding (pause intervals). PIR, at both doses studied, antagonized the effects of LSD, DOM and QUIP on reinforcements and pause intervals as reflected by increases in ED_{50} values for reinforcements (see Table).

ED_{50} for Reinforcements

	Alone	+40 PIR	+160 PIR
LSD (µg/kg)	85	150	363
DOM (mg/kg)	.47	2.82	6.44
QUIP (mg/kg)	1.18	4.35	5.21
LIS (µg/kg)	31.5	42.1	22.6

The effect of LIS on reinforcements was not antagonized by either dose of PIR while its effect on pause intervals was antagonized to some extent. This is in contrast to the antagonism of the behavioral effects of LIS by other 5HT antagonists (pizotifen and metergoline). These data suggest that the actions of LSD, DOM and QUIP in disrupting operant behavior may be mediated at least in part through $5HT_2$ receptors. In contrast, the disruption of behavior by LIS appears not to be mediated to any great extent by these receptors. However, the functional significance of brain $5HT_2$ receptors is poorly understood at this time. Clarification of the role of these receptors in central neural processes that control behavior may aid in understanding the mechanism(s) by which hallucinogens disrupt these systems. (Supported by NIDA grant DA01836.)

340.6 STIMULUS DISCRIMINABILITY AND LEARNING FOLLOWING MEDIAN RAPHE LESIONS. D. Wirtshafter, W. Montana and K. E. Asin. Dept. Psych. Univ. IL at Chicago, Chicago, IL 60680.

In the past we have presented evidence suggesting that electrolytic lesions of the median raphe nucleus result in a syndrone of behavioral disorders resembling those seen after damage to the hippocampus. For example, both median raphe and hippocampal lesions have been reported to increase open field activity, impair performance of an 8-arm radial maze task, impair timing on an operant DRL task, abolish spontaneous alternation, impair performance of a step-through passive avoidance task, and impair the acquisition of a T-maze delayed alternation response. Furthermore, we have found that these effects do not appear to result from serotonin depletion.

In the current study we sought to examine in greater detail the nature and neurochemical substrate of the behavioral deficit produced by electrolytic median raphe lesions by examining the effects of these lesions on the acquisition of three food reinforced T-maze brightness discrimination tasks of varying difficulty. Testing was conducted in a T-maze with frosted Plexiglass floors under which were mounted lights whose brightness could be adjusted by means of dimmer circuitry. Sham operated and median raphe lesioned rats were trained on either an easy, an intermediate, or a difficult discrimination habit, as defined by the difference in light intensity between the correct and incorrect arms. Animals with raphe lesions were able to acquire the easy discrimination within normal limits, were moderately impaired on the intermediate task, and were severely impaired on the acquisition of the difficult task. These results are similar to those reported after hippocampal damage. Subjects who had been trained on the easy condition were then switched to first the intermediate, and then the difficult task. Under these conditions, both control subjects and those with median raphe lesions were able to rapidly achieve criterion performance on the difficult discrimination suggesting that the impairment seen in rats with raphe damage can be ameliorated by directing their attention to the relevant stimulus aspects of the testing situation.

In order to investigate whether serotonin is involved in the effects reported above, we examined acquisition of the intermediate brightness discrimination in rats with injections of 5,7-dihydroxytryptamine, or its vehicle, into the median raphe. In contrast to the impairment seen after electrolytic lesions, animals with neurotoxin injections tended to acquire the discrimination more rapidly than controls. These results suggest that the deficit seen after electrolytic lesions must reflect damage to nonserotonergic elements within the median raphe.

340.7 DOPAMINE AND MOTOR LEARNING IN KORSAKOFF'S PSYCHOSIS. R.G. Mair and W.J. McEntee. Neurology Service, V.A. Medical Center, Providence, RI 02908.

Korsakoff's psychosis is an organic amnestic syndrome attributed to chronic alcoholism and thiamine deficiency and associated with with pathologic lesions in the medial brainstem and diencephalon. We have reported evidence that central monoaminergic activity is diminished in this disease. Earlier studies of Korsakoff patients in our laboratory have shown significant correlations between the concentration of CSF MHPG, the primary brain metabolite of norepinephrine, and neuropsychometric measures of amnesia. On the other hand we have observed that, CSF HVA, the primary brain metabolite of dopamine, is correlated with performance on digit-symbol substitution tasks (DSST) but not with measures of amnesia. We now report data describing the performance of a group of 8 Korsakoff patients on two other motor learning tasks, rotary pursuit (RP) and mirror tracing(MT). Our results show significant intercorrelations between performances on DSST, RP, and MT but not between any of these motor learning tasks and measures of amnesia. Both RP (r= .84) and MT (r= .89) performance correlated significantly with levels of CSF HVA but not of CSF MHPG. These data support the hypotheses that central dopaminergic systems mediate motor learning and that the acquisition of motor and verbal information depend upon different neural substrates.

340.8 OPERANTLY CONDITIONED ENDURANCE TRAINING INCREASES RAT BRAIN CATECHOLAMINES WHILE DECREASING RECEPTOR DENSITIES, J. M. de Castro and G. Duncan. Dept. of Psychology, Georgia State Univ., Atlanta, Ga. 30303 (Spon: D. Edwards)

Chronic aerobic exercise has been shown to not only improve physiological fitness but also to improve mood. It has been hypothesized that the antidepressant effects of endurance exercise result from changes in neurochemistry similar to those produced by antidepressant drugs and electroconvulsive shock treatment, in particular to an alteration in the catecholamine systems. Rats were trained to run for food reinforcement on a variable ratio schedule in running wheels. Yoked control rats were also allowed to run but were not specifically reinforced for running. The animals ran 5 days per week for 8 weeks and were sacrificed 48 hours after the last running session. The brains were assayed for norepinephrine and dopamine concentrations and beta-adrenergic and dopaminergic receptor densities. Changes in norepinephrine concentrations and beta-adrenergic receptor densities were not significantly different between endurance trained and yoked control rats. Dopamine concentrations were significantly higher while dopamine receptor densities were significantly lower in the endurance trained group. These results suggest that endurance training elevates dopamine secretion and consequently produces a compensatory down-regulation of dopamine receptor sites. The relationship of these changes to motor behavior and the antidepressant effects of exercise are discussed.

340.9 CONTROL OF HIPPOCAMPAL β-RECEPTORS IN LEARNED HELPLESSNESS. J.O. Johnson* and F.A. Henn. Department of Psychiatry and Behavioral Science, SUNY at Stony Brook, Stony Brook, NY 11794.

Work previously reported showed that groups of animals, who develop "learned helplessness" (LH), an animal model of depression, have increased levels of β-adrenergic receptor in the hippocampus (ASN Abst. 65, 1982). We have been able to examine the β-receptor density in individual rats using the radioligand iodocyanopindolol (ICYP). This technique has allowed us to directly compare quantifiable LH behavior with the numbers of β-receptors per animal in four regions of the rat brain; hypothalamus, septum, hippocampus, and anterior neocortex. These areas are part of the limbic system which has been implicated in affective disorders in human and a variety of basic behaviors in rats. The primary noradrenergic input to the limbic system consists of a pathway beginning in the locus coeruleus, sending fibers to the hypothalamus and the septum, to terminate diffusely in the cortex and directly in the hippocampus.

The degree of LH behavior can be assessed by determining the number of correct responses in a test situation. Those rats showing extreme LH behavior following conditioning exhibit the greatest increase in β-receptors compared to animals which do not develop LH behavior. A moderate degree of LH behavior results in a moderate rise of β-receptor levels in the hippocampus. The correlation between LH behavior and increased β-receptor levels in the hippocampus is significant ($r=.92$, $p<.05$) suggesting that the increase in β-receptors is directly related to the observed behavioral change. To test the hypothesis that β-receptor changes in the hippocampus are causally related to behavioral changes seen in LH, attempts were made to dissociate the behavior from receptor changes. One simple case was found where this occurred. If animals are conditioned to develop LH and not reinforced after the initial conditioning they will revert to normal behavior after 2-3 weeks. These "cured" rats are found to retain marked elevations of β-adrenergic receptors in the hippocampus. Thus, these β-receptor changes are not sufficient to cause the behavioral changes seen. In addition, septum, hypothalamus, and anterior neocortex showed no significant changes in β-receptor density.

These results indicate that the regulation of the hippocampal β-receptors is different than the β-receptors of other brain regions rostral to the locus coeruleus. This regulation is correlated with the onset, but not the spontaneous termination of LH behavior and is specific for one CNS region. This suggests that something other than the rate of norepinephrine release such as other neurotransmitter systems or hormonal control is involved in regulating hippocampal β-receptor levels.

340.10 BEHAVIORAL MODIFICATION OF DORSAL RAPHE (DRN) ACTIVITY, PONTOGENICULOOCCIPITAL (PGO) WAVES AND THE ULTRADIAN SLEEP CYCLE. R. Lydic, R.W. McCarley, and J.A. Hobson Laboratory of Neurophysiology, Harvard Medical School, Boston Ma 02115

Using intact and unanesthetized cats, we have examined the possibile contribution of DRN to control of behavioral state or incidental state signs by manipulating the period length of the ultradian sleep cycle while recording DRN activity.

Immediately preceeding the DRN and electrographic recordings, cats were placed in a slowly moving (0.05 M/s) treadmill for 12 to 16 hrs. or spent an equivalent amount of time in their home cage. Single unit recordings have been obtained using microwire electrodes from nine DRN cells across a total of 315 sleep cycles following the two behavioral pretreatments. Behavioral state, PGO waves and DRN discharge have been analyzed by averaging across multiple sleep cycles. Sleep cycle period length (Tau) was defined from the end of D in one cycle through D end in the subsequent cycle.

Sleep cycles were organized into four groups: short (Tau < 15 min.), regular (Tau = 15-30 min.), extended (Tau = 31-45 min.), and long cycles (Tau > 45 min.). Cats exposed to the treadmill task spent less time in W or S, more time in D-sleep, and displayed enhanced regularity of the corresponding DRN discharge and PGO wave profiles. During the regular cycles there was a smooth decline in the time course of DRN activity inversely correlated with the onset of PGO waves. Cats not exposed to the treadmill task exhibited more variability in Tau and a larger number of extended and long cycles. Following the treadmill task, time series analyses (linear/nonlinear least squares period analysis) revealed statistically significant ($p<0.001$) ultradian rhythms of behavioral state, DRN discharge, and PGO waves with period lengths in the 17 to 19 min. range. Similar analyses of recordings which did not follow the treadmill task revealed longer Taus (32-37 min.) for all three variables ($p<0.01$).

Behaviorally induced alterations in sleep cycle Tau were accompanied by similar changes in Tau of both DRN and PGO wave activity. The phase relationships and degree of coupling between these three rhythms are consistent with the hypothesis that the DRN modulates both behavioral state and physiological trait variables.

Supported by grants: MH-13923; RSDA:MH-280(RWM); NRSA:MH-14275(RL).

340.11 MODIFIED METHOD FOR ^{3}H-APOMORPHINE BINDING IN RAT BRAIN TISSUE. C. H. Misra, I. Irabor* and R. C. Smith. Biological Psychiatry, Texas Research Institute of Mental Sciences and Department of Pharmacology, Baylor College of Medicine, Houston, Texas, 77030.

The dopamine agonist apomorphine has been used to investigate dopamine receptors; one use has been to distinguish between the single dopamine receptor hypotheses with agonist and antagonist states (Life Sci., 17:933, 1975), vs. a multiple receptor hypothesis (Proc. Nat'l. Acad. Sci. 75:1153, 1978). Some of the contradictions of the results of prior studies may have been caused by problems in ^{3}H-apomorphine binding; the parameters of apomorphine binding varied greatly in different studies and appear to be different in calf vs. rat striatum (Life Sci. 23:495, 1978; Nature 27:278, 1978). The binding assay conditions for ^{3}H-apomorphine were not consistent. Our own studies have investigated and established optimal conditions for ^{3}H-apomorphine binding in rat striatum. We have found that 50mM Tris-HCl buffer pH 7.5 (instead of pH 7.4) is the most appropriate buffer for ^{3}H-apomorphine binding assay. The presence of cations in buffer was found to inhibit the binding. We have obtained maximum binding by incubating the assay mixture (consisting of 4nM ^{3}H-apomorphine and 0.3 to 0.4mg of protein/ml) at 25°C (instead of 37°C) for 10 minutes. By using this modified assay procedure, we have found the potency of various drugs competing against ^{3}H-apomorphine binding in rat striatum to be in the following order: apomorphine> dopamine> butaclamol> domperidol> droperidol> spiroperidol.

340.12 INFLUENCE OF IONTOPHORETICALLY APPLIED NOREPINEPHRINE AND STIMULATION OF LOCUS COERULEUS ON GRANULE CELL RESPONSES TO PERFORANT PATH STIMULATION. Jonathan Winson and Dennis Dahl*, The Rockefeller University, New York, N.Y. 10021

In rats anesthetized with Chloropent, norepinephrine (NE) was applied iontophoretically (.5 or 1 M, 25-200na) from multibarrel micropipettes at successive dorso-ventral positions along the dendritic tree of the granule cells of the dentate gyrus, at the cell body and in the sub-cellular hilar region. At each position recordings were made of the field potentials evoked by stimulation of the medial perforant pathway. NE (but not vehicle control) reduced the slope and magnitude of the field potential recorded at the site of termination of the medial perforant path (evoked synaptic potential or ESP) in a dose dependent manner, but did not affect the field potential at other positions along the dendrites. There was no effect of NE applied at the granule cell layer. Application of NE in the hilar region increased the field potential population spike recorded at this level. In other experiments, recordings were made simultaneously of the ESP and the field potential in the cell body layer or hilus. Reduction of the ESP slope was not accompanied by a corresponding reduction of the slope of the field potential in either of these regions.

The locus coeruleus was stimulated utilizing 6-12 pulses at 50 Hz, applied 50 msec prior to the application of a pulse to the perforant path. The effect of locus coeruleus stimulation was to reduce the ESP and increase the population spike.

These results suggest that NE may act at two sites, the middendritic region and the sub-cellular hilus, to affect granule cell responsivity to medial perforant path stimulation. This will be discussed in terms of previous findings regarding neuronal transmission from the perforant path through the dentate gyrus in freely behaving animals.

(Supported by National Institute of Mental Health Research Scientist Development Award 5-K02-MH00232, National Science Foundation Grant BNS 80-13034, and a grant from the Harry F. Guggenheim Foundation to J. Winson

341.1 SYNAPTIC CHANGES IN MEDIAL PREFRONTAL CORTEX OF HALOPERIDOL-TREATED RATS. F.M. Benes*, P.A. Paskevich*, J. Davidson* and V.B. Domesick. Dept. of Psychiatry, Harvard Medical School, and the Mailman Research Center, McLean Hospital, Belmont, MA 02178.

A morphometric analysis of the medial prefrontal cortex (MPF) of control (N = 4) and haloperidol-treated (N=4) rats was performed at the light and electron microscopic (EM) level to determine whether structural rearrangments can be induced after chronic treatment (16 wk) with an agent which blocks dopamine receptors. Layer VI of MPF was studied since it receives tegmental A10 dopamine innervation. Toluidine blue-stained thin sections (1 μm) examined at the light microscopic level revealed no difference between the two groups for either neuronal cell size or density. At the EM level, samples of both axosomatic and axodendritic terminals were obtained separately. The number of terminals per cell or dendrite profile, the terminal size, synaptic vesicle density and type of membrane specialization (symmetric, SMS, asymmetric, AMS, or no specialization, NMS) for each terminal were determined. There was a significant reduction ($p = 0.003$) in the number of axosomatic synapses per cell for the haloperidol-treated group, while terminal size and vesicle density were unchanged. The number of axosomatic synapses per cell showed a bimodal distribution and both peaks showed a uniform left shift in drug-treated animals. Synapses with SMS or NMS accounted jointly for 95% of all axosomatic terminals, and both types were uniformly decreased in the drug-treated group. Axodendritic terminals also showed no change in the terminal size or vesicle density, but, unlike axosomatic synapses, revealed no reduction in the overall number of terminals per dendrite cross-section. Despite the lack of change in number of axodendritic terminals, the haloperidol-treated group showed a preferential loss (24%) of terminals with AMS and a 100% increase of those with NMS. The AMS terminals were preferentially localized to three distinct small calibre peaks of dendrites which are likely spines. One of these peaks was completely absent from the haloperidol-treated animals, while the other two were markedly reduced (approximately 50%). The loss of these small calibre peaks caused the overall size of dendrites in the drug-treated group to appear to be twice that of controls ($p = 0.0002$). These data suggest that haloperidol may induce a variety of alterations in both axosomatic and axodendritic synaptic arrangements in layer VI of rat MPF. [Supported by NIMH Grants 1K01-MH004253-0 and MH31154-06].

341.2 SYNAPTIC ULTRASTRUCTURE OF GOLDFISH CRANIAL MOTOR NUCLEI RELATED TO ELECTRICAL-CHEMICAL SYNAPTIC TRANSMISSION. J.T. Hackett and A. Buchheim*. Dept. of Physiol., Univ. of Virginia, School of Medicine, Charlottesville, VA 22908

A single impulse in a Mauthner cell can produce a unilateral contraction of the body and a bilateral contraction of the mandibular, extraocular, and opercular muscles. Several neurons, each postsynaptic to both Mauthner axons, relay impulses to the cranial motoneurons on one side (Hackett & Faber, Neurosci. 8, 317, '83). We now report the identification of the cranial relay neuron (CRN)-motoneuron synapse and the demonstration of the modes of synaptic transmission.

Experiments were performed on goldfish 10-15 cm in length that were respired through the mouth with aerated water and were anesthetized with pentobarbital. The connections of the CRN-axons were examined with light and electron microscopic techniques, and the mechanisms of synaptic transmission were determined by intracellular recording with two microelectrodes. Horseradish peroxidase (HRP) and Leucifer yellow were injected into CRN-axons to demonstrate anatomically that they diverge to several motor nuclei, as well as, to many motoneurons within one nucleus. Retrograde transport of the enzyme was used to label the cranial motoneurons.

Under the electron microscope, CRN-terminals were identified by the granular appearance of the electron-opaque polymer formed enzymatically with HRP. The labeled motoneurons had the polymer enclosed in lysosomes. Before making synaptic contact, the CRN-axon became unmyelinated. The CRN-terminals contained many presynaptic vesicles which concentrated the HRP reaction product. Active zones were evident, as well as, a synaptic cleft of about 50 nM. At some synapses, both gap junctions and presynaptic vesicles were found. Leucifer yellow did not seem to pass through the gap junctions. Composite postsynaptic potentials in a trigeminal motoneuron were evoked by intracellular stimulation of a CRN-axon. The earliest component had an apparent synaptic delay of 0.26 msec. and had a peak amplitude that was not depressed by repetitive stimulation. A second component was distinguished by its larger peak amplitude which was reduced by repetitive stimulation at a frequency of 2 Hz. Antidromic action potentials failed to be transmitted from motoneurons to the CRN-axons.

Thus, both electrical and chemical transmission probably occur at the CRN-motoneuron synapse. Since the short transmission times and extensive divergence of the Mauthner cell network account for the rapidity and security of the elicited behavioral response, we propose that the presence of chemical synaptic transmission provides for an adaptive system.
(Supported by NSF Grant BNS 81-12742)

341.3 STRUCTURE AND PHYSIOLOGICAL PROPERTIES OF 5-HT SYNAPSES MADE BY INDIVIDUAL NEURONS IN CULTURE. P. Drapeau*, D.P. Kuffler and J.G. Nicholls, Dept. of Neurobiology, Stanford Med. Sch., Stanford, CA 94305.

Individual neurons of known function dissected out of the CNS of the leech have been shown to survive in culture and maintain their distinctive electrical, biochemical and morphological characteristics. Moreover, by 3 to 5 days electrical and chemical synaptic interactions develop in a predictable manner between some cells but not others.

In the present experiments we have examined by electron microscopy chemical synapses formed by Retzius cells upon P cells in culture: here the transmitter has been identified as 5-HT and shown to be released in quantal units of fixed size (Henderson, Kuffler, Nicholls & Zhang, 1983, J. Physiol., in press). At the time that transmission is established well-defined synaptic specializations are observed, particularly at sites where fingers of the Retzius cell invaginate the P cell. In these regions the membranes of the two cells are thickened and farther apart than usual. Within the widened synaptic cleft, electron dense material is apparent. Dense core and clear vesicles are aggregated in the presynaptic Retzius cell terminals close to the membrane. No comparable aggregates of vesicles are seen in the P cells, which do not produce synaptic potentials on the Retzius cell. Vesicles in the Retzius cell accumulate a marker such as HRP if it is present in the culture medium while the cell is depolarized by high concentrations of potassium.

These two cells in direct apposition having numerous synaptic specializations, situated not on distant processes but close to the soma, offer advantages for studying the turnover of vesicles and changes in structure under different physiological circumstances.

341.4 ACETYLCHOLINESTERASE IN THE SYNAPTIC BASAL LAMINA OF DAMAGED MUSCLE FIBERS. Lili Anglister and U.J. McMahan. Dept of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

Acetylcholinesterase (AChE) in skeletal muscle is concentrated at neuromuscular junctions (NMJs) where at least some is adherent to the portion of the muscle fiber basal lamina sheath within the synaptic cleft. After muscle damage that results in phagocytosis of all cellular components of NMJs but spares basal lamina sheaths, AChE remains bound to the basal lamina for months. Of the several molecular forms of AChE in muscle, there is evidence that those that are the largest and have collagen-like tails are associated with the basal lamina at normal NMJs. It has been postulated that the tails help to anchor these forms in place. The aim of the experiment described here was to determine what forms of AChE are bound to the synaptic basal lamina after muscle damage.

The cutaneous pectoris muscle of the frog, which has the usual molecular forms of AChE, was damaged by freezing it in situ which caused disintegration of all cells--myofiber, nerve terminal and Schwann cell--at all NMJs. Regeneration of axons was prevented by nerve evulsion and of myofibers by x-irradiation of the frog. Thirty days after damage, when virtually all fragments of cells discernible by EM had been phagocytized, the "muscle," now containing only empty myofiber basal lamina sheaths, was removed for study. Staining for AChE showed that the enzyme was still highly concentrated in the synaptic portion of the sheaths. Biochemical analysis of the synaptic region of the empty sheaths revealed only a small form of AChE having a sedimentation coefficient of 4.5S, which is characteristic of globular (tailless) forms isolated from other animals. Extrasynaptic regions of the sheaths had no detectable AChE activity. The junctional AChE was loosely bound to the sheaths; it was solubilized simply by homogenization in isotonic saline. Even perfusion of isolated freeze-damaged muscles with frog Ringer resulted in decreased AChE staining; the perfusate contained AChE.

We do not yet know whether the globular form found in the synaptic basal lamina after freeze damage is a constituent of basal lamina at normal NJMs in the frog; it may have been derived from disrupted tailed forms. Our results demonstrate, however, that AChE in the synaptic basal lamina need not have a collagen-like tail to remain in position. It can be held in the basal lamina by associations involving the globular region of the molecule.

(Sponsored by NIH grant NS 14506 and a Rothschild Foundation Postdoctoral Fellowship to L.A.)

341.5 FORMATION OF SYNAPTIC JUNCTIONS DURING POSTNATAL DEVELOPMENT IN CEREBELLAR CORTEX, D.M.D. Landis, Neurology Service, Massachusetts General Hospital, Boston, MA 02114

In mature animals, the excitatory synapse between parallel fibers and Purkinje cells is located exclusively on dendritic spines. The synaptic junction has asymmetric, electron-dense fuzz in thin sections, and in freeze-fractured preparations the extracellular half of the postsynaptic membrane is characterized by an aggregate of particles, coextensive with the synaptic cleft. However, the first morphological evidence of junction formation between parallel fibers and growing Purkinje cell dendrites in the nascent molecular layer during the first two postnatal weeks is located on dendritic shafts, not spines, and consists of electron-dense submembrane fuzz lining the apposed membranes, usually associated with a collection of synaptic vesicles in the axon. Careful examination of freeze-fractured preparations reveals that the initial shaft junctions are not associated with particle aggregates on the extracellular half of the membrane. Their structure is therefore different from that of mature synaptic junctions.

In distal portions of the growing dendritic arborization, there are small clusters of 5-15 particles on the extracellular membrane half. The constituent particles resemble those at mature synaptic junctions, but in fortuitous cross-fractures it is clear that the clusters do not coincide with junctions. In addition, there are very infrequent aggregates of many particles on the dendritic shaft, some of which are apparently coextensive with junctions. These large aggregates do not occur in adults, and are therefore transient.

In serial thin sections of growing dendritic arborizations, there are spinous evaginations without associated junctions. This may indicate that spine formation can occur at non-junctional sites, but it is also possible that these evaginations are simply growing branch points.

We believe that initial electron-dense junctions on the shaft and the small particle clusters develop independently. The constituent particles of the clusters usually assemble into aggregates on spinous evaginations, but aggregate formation can also occur at sites other than spinous evaginations. The precise manner in which the initial junctions are replaced by junctions on spines with coextensive aggregates is still uncertain. The physiological role of the initial junction may include recognition and adhesion; its capacity for synaptic transmission is unknown.

341.6 FREEZE-FRACTURE STUDY OF MEMBRANE STRUCTURE IN THE CA1 REGION OF RAT HIPPOCAMPUS. K.M. Harris and D.M.D. Landis (SPON: E. Frank) Neurology Service, Massachusetts General Hospital, Boston, MA

Several distinct classes of synaptic interaction have been identified in the s. radiatum of the CA1 hippocampal region. Schaffer collateral axons form excitatory synapses with CA1 pyramidal cells, which correspond to junctions on spines with asymmetric membrane specializations. These dendritic spines in aldehyde-fixed, freeze-fractured preparations are characterized by an aggregate of particles associated with the extracellular half of the fractured membrane. The aggregate is coextensive with a widened synaptic cleft, and probably corresponds to the site of postsynaptic electron-dense fuzz seen in thin sections. Some of these particle aggregates are annular.

Interneurons establish inhibitory synapses with the CA1 pyramidal neurons; these probably correspond to synaptic junctions on perikarya and dendrites with symmetric membrane specializations. In fractured tissue, we encountered axonal boutons apposed to the dendritic shaft surface, without evident specialization of particle distribution on the extracellular membrane half. These may represent inhibitory synapses. The extracellular half of the fractured perikaryonal plasmalemma appears virtually devoid of particle aggregates, though symmetric synaptic junctions are known to occur on these cells. Pyramidal cell perikarya had aggregates of particles on the cytoplasmic membrane half, some of which were coextensive with membranes in the subjacent cytoplasm and so are likely to be specializations at the sites of subsurface cisterns.

Comparatively few particle aggregates are present on the extracellular half of the dendritic shaft membrane; in fortuitous cross-fractures, aggregates could be seen to be coextensive with axonal varicosities. These shaft aggregates may correspond to asymmetric synaptic junctions visualized in thin sections.

Thus, excitatory synapses in this region have aggregates of particles arrayed on the extracellular half of the postsynaptic membrane, like those described previously at excitatory synapses in cerebellar cortex, olfactory bulb, and anteroventral cochlear nucleus. The synapses with an inhibitory action in the CA1 region have no apparent specialization of intramembrane particle distribution in the postsynaptic membrane, and so are similar to inhibitory synapses in cerebellar cortex, olfactory bulb, and anteroventral cochlear nucleus.

341.7 CHARACTERIZATION OF AN ATP-DEPENDENT SYNAPTOSOMAL Ca^{++} PUMP, * USING ANTISERA AND MONOCLONAL ANTIBODIES. S.Y. Chan, E.J. Hess, H. Rahamimoff and S.M. Goldin. Dept. of Pharmacology, Harvard Medical School, Boston, MA 02115.

An ATP-dependent Ca^{++} transport component was identified in a vesicle fraction obtained from osmotically lysed bovine brain synaptosomes. This component was reconstituted into artificial lipid vesicles and purified by an adaptation of the "transport specific fractionation" procedure of Papazian, Rahamimoff and Goldin (PNAS(1979)76,3708). Two major protein components of M_r 230,000 and 94,000 (C230 and C94; respectively) were identified by SDS gel electrophoresis of the purified preparation. C94 corresponded to the major hydroxylamine sensitive [γ-^{32}P]ATP-phosphorylatable protein of synaptosomal vesicles; other transport ATPases are known to be phosphorylated in a similar manner.

Antiserum (AS) and subsequently monoclonal antibodies (MAbs) were raised against this preparation. By solid phase radioimmunoassay against the synaptosomal vesicles, the 50% titer points of AS and MAb ascites fluid was typically 1/1,000 and 1/100,000, respectively. By competitive RIA, substantial cross-reactivity of both AS and MAbs was observed towards bovine brain axonal membranes (axolemma) prepared by the procedure of DeVries et al. (Brain Res. (1978)147, 339). However, no cross-reactivity against the Ca^{++} pumps of bovine muscle sarcoplasmic reticulum or bovine erythrocytes was exhibited. At least 50% of the ATP-dependent Ca^{++} uptake activity was specifically immunoprecipitatable, from both native synaptosomal vesicles and after its reconstitution into artificial lipid vesicles using MAb and Protein A of S.aureus.

The Western Blot procedure was used to detect the specific interaction of AS and MAbs with synaptosomal vesicle proteins that had been subjected to SDS gel electrophoresis. AS bound to C230 strongly, to C94 to a much lower degree, and also interacted with several proteins of lower molecular weight. In contrast, the MAbs tested all bound primarily to C230 alone.C94 in isolation was obtained by its elution from SDS gels of the purified synaptosomal Ca^{++} transport component and used as antigen to prepare a specific AS. The resulting AS to C94 bound predominantly to C230 on Western Blots, indicating that C230 and C94 were immunologically related.

These results provide evidence that synaptosomes contain a nerve tissue-specific Ca^{++} pump that is immunologically distinct from the Ca^{++} pumps of erythrocytes and muscle sarcoplasmic reticulum. C94 is a functional component of this pump. C230 copurifies with, is immunologically related to, and could conceivably be a dimer of C94. Supported by grants to SMG by the NIH (NS 16475), the McKnight Foundation, and the Searle Scholars Program. SYC is a Pharmaceutical Mfr's Ass'n Postdoctoral Fellow.

341.8 INTRINSIC INTERACTION OF TUBULIN ASSOIATED CALMODULIN DEPENDENT KINASE WITH TUBULIN IN BRAIN CYTOSOL. J. R. Goldenring, J. E. Casanova,* R. E. Larson,* and R. J. DeLorenzo. Dept. of Neurology Yale U. School of Medicine, New Haven, CT 06510.

The interactions between Ca-calmodulin (Ca-CaM) and tubulin may mediate important dynamic functions in neurons. A Ca-CaM dependent tubulin kinase from brain cytosol has recently been isolated away from CaM and endogenous substrate tubulin (BBRC 108: 481). The tubulin associated CaM dependent kinase (TACK), purified to homogeneity through Fractogel TSK HW-55 chromatography, contained two subunits of 52,000 (rho) and 63,000 (sigma) daltons. Both of these subunits bound, CaM, autophosphorylated, and displayed pI's between 6.7 and 7.2 (Trans. Amer. Soc. Neurochem. 14: 186). We now report the isolation from brain cytosol of a complex containing alpha and beta-tubulin and the TACK subunits, demonstrating the association of this CaM dependent kinase with its endogenous substrate.

Rat brain cytosol was chromatographed on DEAE-cellulose. A 500 mM NaCl elution contained all of the cytoplasmic tubulin and endogenous Ca-CaM stimulated tubulin kinase activity. DEAE-isolated tubulin kinase activity was chromatographed on Fractogel and essentially all of the tubulin kinase activity eluted as a single high molecular weight peak. Rechromatography of this peak on either Fractogel or Sephacryl S-300 yielded only a single high molecular weight peak, demonstrating the integrity of the complex. The protein staining pattern revealed both tubulin and also the TACK subunits. The Fractogel-isolated complex demonstrated Ca-CaM dependent tubulin kinase activity and phosphorylation of proteins with molecular weights and pI's identical to the rho and sigma subunits of TACK. As with the TACK enzyme, the tubulin-enzyme complex phosphorylated beta-tubulin equally on both serine and threonine residues.

In the presence of chelator and high salt, TACK could be dissociated from tubulin. Passage over DEAE-cellulose then yielded kinase activity in the void volume while tubulin adhered to the resin. The kinase activity that passed through the column adhered to CaM-affinity resin. The kinase eluted from the resin with chelator contained two peptide species of 52,000 and 63,000 daltons which were identical to the rho and sigma subunits of TACK based on 2-dimensional tryptic peptide mapping.

The results demonstrate a tight association between TACK and tubulin in brain cytoplasm. The functional status of this kinase in association with tubulin was further investigated by comparison with the microtubule associated tau proteins which bind CaM (FEBS Lett. 132:137). We found that the tau_1 protein was homologous with the rho subunit of TACK by peptide mapping. Thus, TACK may represent an intrinsic Ca-CaM dependent regulatory system for maintenance of tubulin-microtubule dynamics.

341.9 IDENTIFICATION OF CALMODULIN DEPENDENT KINASES IN SYNAPTIC VESICLE SYNAPTIC MEMBRANE, AND POSTSYNAPTIC DENSITY PREPARATIONS HOMOLOGOUS WITH TUBULIN ASSOCIATED CALMODULIN DEPENDENT KINASE.
R. J. DeLorenzo and J. R. Goldenring. Dept. of Neurology, Yale University School of Medicine, New Haven, CT 06510.

Tubulin and calmodulin (CaM) play important roles in the organization and regulation of nerve cells. Ca-CaM dependent tubulin kinase activity from rat brain cytosol has recently been isolated (BBRC. 108:481, 1982). The tubulin associated CaM dependent kinase (TACK), purified to homogeneity, contains two CaM binding, autophosphorylating subunits of 52,000 (rho) and 63,000 (sigma) daltons, both of which possess pI's between 6.7 and 7.2. We now report that homologous tubulin and microtubule associated protein kinase systems exist in brain membrane, synaptic vesicle, and postsynaptic density fractions. TACK proteins were compared by five criteria: 1) migration on 1-dimensional SDS-PAGE; 2) isoelectric points; 3) autophosphorylation; 4) calmodulin binding; and 5) tryptic peptide maps.

Tubulin kinase activity has been observed in synaptic vesicle (Burke and DeLorenzo. J. Neurochem. 38:1206, 1982), synaptic membrane (DeLorenzo, et al. Prog. Brain Res. 56:255, 1982), and postsynaptic density (Mahler, et al. Prog. Brain Res. 56:27, 1982) preparations. These findings suggest that the TACK system isolated and characterized from brain cytosol may exist in membrane-bound forms in a similar but modified form. Synaptic vesicle (SV) synaptic membrane (SM), and postsynaptic density (PSD) fractions all displayed Ca-CaM dependent phosphoproteins with essentially identical molecular weights and isoelectric points to the rho and sigma subunits of cytosolic TACK. These three fractions also contained CaM binding proteins with similar characteristics to rho and sigma. To specifically investigate the suggested identities of these 50,000 and 60,000 dalton proteins in SV, SM, and PSD preparations with the rho and sigma subunits of TACK, protein stained bands were isolated from 2-dimensional gels and analyzed through 2-dimensional tryptic peptide mapping. The peptide maps demonstrated the presence of proteins in SV, SM, and PSD fractions which were essentially homologous with the rho and sigma subunits of TACK with specific minor modifications. In particular, the rho subunit of TACK was found to be essentially identical by all criteria to the major 52,000 dalton PSD protein.

These observations indicate that the TACK system is present in nerve cells in membrane-bound and unbound forms. The endogenous phosphorylation of tubulin and the rho and sigma subunits of TACK in cytoplasm, SV, SM, and PSD fractions represent a major portion of the phosphorylated 50,000 and 60,000 dalton proteins that were previously designated DPH-L and DPH-M (DeLorenzo, et al. PNAS 76:1838, 1979). The TACK system may play a role in mediating some of the interactions between membrane and the cytoskeleton.

341.10 PURIFICATION AND CHARACTERIZATION OF A CALMODULIN-DEPENDENT PROTEIN KINASE FROM RAT BRAIN.
M. K. Bennett, N. E. Erondu and M. B. Kennedy. Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

Calcium and calmodulin can activate a number of different neuronal enzymes including several distinct protein kinases. We report here the purification of a calmodulin-dependent protein kinase that is highly concentrated in rat brain. The kinase was monitored during the purification by its ability to phosphorylate the synaptic vesicle associated protein, synapsin I. Purification from the soluble fraction involved the following steps: 1) DEAE cellulose chromatography; 2) ammonium sulfate fractionation; 3) calmodulin-Sepharose affinity chromatography; 4) gel filtration chromatography; 5) sucrose density gradient centrifugation. The final product is 95% pure and represents a 290-fold purification over the crude homogenate. The yield of purified kinase indicates that it is a relatively abundant brain enzyme comprising about 0.3% of the total brain protein. The purified enzyme is composed of three subunits that comigrate with kinase activity during the purification steps and are coprecipitated with kinase activity by a specific anti-kinase monoclonal antibody. The three peptides have molecular weights of 50,000, 58,000, and 60,000 and have been termed α, β', and β respectively. The α and β-subunits are distinct peptides, however β' may have been generated from β by proteolysis.

In order to define the subunit composition of the protein kinase holoenzyme, we investigated its hydrodynamic properties. The Stokes radius of the kinase was 95Å, and the sedimentation coefficient was 16.4S. A molecular weight for the holoenzyme of 650,000 was calculated from these values. To determine the relative amounts of each subunit present in the holoenzyme, we made densitometric scans of SDS/polyacrylamide gels stained for protein. For this analysis, we grouped the β subunits and treated them as one. The subunits are present in a ratio of about 3α-subunits to 1 β/β'-subunit. Thus, we postulate that the 650 kD holoenzyme consists of approximately 9α and 3 β/β'-subunits. ^{125}I-calmodulin bound specifically to all three subunits of the enzyme. All three subunits are also autophosphorylated. This latter finding confirms the hypothesis suggested in earlier reports (Kennedy et al, 1983, J. Neurosci. 3:818) that three prominent "substrate" proteins for a calmodulin-dependent protein kinase in brain homogenates are actually autophosphorylated subunits of the calmodulin-dependent protein kinase itself. We have also shown that one of the kinase subunits, the α-subunit, is identical to the "major postsynaptic density protein" described by Kelly and Cotman (1978, J. Cell Biol. 79:173). (Supported by NS17660, 1 T32 GM07616, and a Gordon Ross fellowship.)

341.11 IMMUNOCHEMICAL EVIDENCE FOR A MARKED REGIONAL VARIATION IN THE CONCENTRATION OF THE α-SUBUNIT OF CALMODULIN-DEPENDENT SYNAPSIN I KINASE IN DIFFERENT BRAIN REGIONS.
N. E. Erondu, M. B. Kennedy, and V. Krieger*. Division of Biology 216-76, California Institute of Technology, Pasadena, California 91125.

A number of protein kinases activated by calcium have been described. These calcium-dependent kinases appear to differ in several characteristics--mechanisms of regulation by calcium, substrate specificity, tissue distribution, as well as subunit composition. To aid our studies of the calmodulin-dependent synapsin I kinase that we have recently purified from rat brain (see Bennett et al. this session), we have generated a panel of monoclonal antibodies that specifically recognize it. One of these antibodies (6G9) binds with high specificity and high affinity to the α-subunit of the kinase holoenzyme.

We have used this antibody to quantitate the distribution of the α-subunit in different regions of rat brain. The regions were dissected in the cold, homogenized immediately, and centrifuged at 2000 x g for 10 min. Aliquots of the resultant supernatants (25 to 200 µg) were subjected to SDS-polyacrylamide gel electrophoresis, then transferred onto nitrocellulose paper as described by Towbin et al. (PNAS, USA 76:4350). The paper sheets were incubated with monoclonal antibody, rabbit anti-mouse IgG, and ^{125}I-Protein A, in that order. The ^{125}I-labeled bands were located by autoradiography, cut out and counted in a Gamma counter. The amount of α-subunit in each homogenate was then determined by comparison to a standard curve prepared with pure synapsin I kinase. The limit of detection in this assay was 20 ng of α-subunit/100 µg protein. The results confirmed the high concentration of α-subunit in brain homogenates and also revealed a marked regional variation in its concentration. For example, in one study, whole rat brain homogenate contained 670 ng of α-subunit/100 µg of total protein; hippocampus, 970; cerebral cortex, 850; striatum, 660; olfactory bulb, 480; hypothalamus, 130; thalamus/midbrain, 100; pons/medulla, 78; cerebellum, 66; and pituitary, 31. Low levels of synapsin I kinase enzyme activity (1/10 to 1/3 that of brain) have been detected in homogenates of certain non-neuronal tissues (Kennedy and Greengard, PNAS, USA 78:1293). However, we have detected no α-subunit in these tissues by the immunochemical method. Thus, the synapsin I kinase holoenzyme purified from rat brain appears to be unique to neural tissue and concentrated in certain brain regions relative to others. It may therefore mediate a response to calcium that is a specialized characteristic of certain neuronal cell types. (Supported by NS 17660, and by a Gordon Ross fellowship.)

341.12 THE ISOLATION OF POSTSYNAPTIC RECEPTORS FROM THE BRAIN: ELECTRON-MICROSCOPIC STUDY AND RECEPTOR BINDINGS.
Y. Hama*, H. Kishimoto, S. Yokoi* and S. Yagishita*, Dept. of Psychiatry, Yokohama City Univ. Sch. of Med., Minami-ku, Yokohama 232, Japan, and Dept. of Pathology, The Kanagawa Rehabilitation Center, Kanagawa 243-01, Japan.

Ultrastructures of membrane preparations from rat brains were examined by electron-microscope. The membranes of cerebral cortex, prepared as described by Zukin et al. (1974), were found to contain much myeline fragments. At high magnification postsynaptic receptors were scarcely observed in this membrane preparation.

The subfractionation of the crude mitchondrial fraction by the dencity gradient method according to De Robertis, separated postsynaptic receptors from both myeline fraction and mitchondrial fraction. [^{3}H]Dopamine bound in saturable fashion to the postsynaptic receptor fractions from the cerebral cortex and the basal ganglia of rat obtained by this method. This [^{3}H]dopamine binding was displaced by nonradioactive dopamine and antipsychotic drugs. The association of [^{3}H]dopamine binding showed that binding reached equilibrium after about 40 min, and the dissociation occured with a half-life of about 10 min. Electron-microscopically postsynaptic receptors were not found in nuclear fraction and microsomal fraction separated by De Robertis' method, but [^{3}H]dopamine bound to these fractions with high affinity. The bindings were saturable and displaced by nonradioactive dopamine. Other [^{3}H]labeled ligands such as [^{3}H]5-HT, [^{3}H]GABA, [^{3}H]glycine bound not only to postsynaptic receptor fraction but also to nuclear and microsomal fractions.

In order to examine receptor properties or relations between psychiatric diseases and receptor functions, it is important to isolate and purify the postsynaptic receptors.

342.1 PROTEINS RAPIDLY TRANSPORTED TO THE SYNAPSES OF THE GIANT NEURON R2 OF APLYSIA CALIFORNICA, R.T. Ambron, S. Schacher* and H. Den*. Anatomy and Cell Biology and Center for Neurobiology and Behavior, Columbia University, P&S, New York, NY 10032.

In neurons, the cell body is responsible for the synthesis of macromolecules. Consequently, membrane proteins destined for the distal axolemma and synapse must be selectively exported from the cell body and then transported along the axon. During transport, proteins required for axonal functions must be segregated from those to be transported to terminals. In an effort to understand how sorting occurs, we are studying the distribution of newly-synthesized proteins in the various regions of the giant neuron R2. R2's cell body resides in the abdominal ganglion and its major axon courses through the right connective to the head ganglia where it divides, sending branches into the parapodial nerves. Ultimately the axons synapse on mucous glands in the skin. Electrophysiological experiments indicate that R2 is the only neuron in the abdominal ganglion with such an axon distribution. This was confirmed by removing the central nervous system from the animal and placing the abdominal ganglion in a separate chamber containing ^{35}S-methionine. After 5h the label was washed out and the tissue maintained for 50h. Radioautography revealed that each peripheral parapodial nerve had only one labeled axon. Using the same labeling protocol, we sectioned the entire nervous system into sequential 2mm segments. When the distribution of the labeled proteins was determined, we found that rapidly transported proteins had accumulated at the distal end of the parapodial nerves during the 50h "chase". Nineteen labeled proteins, ranging in mw from 30-200k, were resolved in this region by polyacrylamide gel electrophoresis: three of these correspond to major glycoproteins previously shown to be rapidly transported in R2. Of the 19 proteins, 15 were also found in more proximal regions of the axon and therefore are believed to have been deposited along the axon during transport. The other four proteins were unique to the distal axon and are presumably en route to the synapse.

R2's cell body can be maintained in culture where it rapidly extends neurites. We have compared ^{35}S-met-labeled proteins transported into growing neurites with those that accumulate in the peripheral nerves as described above. Most of the proteins are found in both, but two of the presumptive synaptic and four of the axonal proteins are not present in the neurites. We are presently growing R2 in the presence of isolated mucous glands to see if synaptic contact is made and, if so, whether the above proteins are then induced in vitro.

342.2 RAPIDLY TRANSPORTED PROTEINS OF NERVE GROWTH CONES AND SYNAPTIC ENDINGS ARE MARKEDLY DIFFERENT. P. Simkowitz* and K.H. Pfenninger, Dept. of Anat. & Cell Biol., Columbia Univ. P&S, New York, NY 10032.

The proteins rapidly transported to the distal tips of growing optic axons of albino rat fetus (16 days gest.) have been compared to their counterparts in the optic nerve terminals of the adult. The eyes of several fetus were exposed by transuterine microsurgery. Each was injected with ~200 µCi of L-[^{35}S] methionine in a volume of ~10 nl. After 4h survival, the diencephala and mesencephala were removed, mixed with unlabeled fetal brains (as carrier) and subjected to subcellular fractionation for the isolation of nerve growth cone particles (GCPs;Ellis et al., Neurosci. Abst., 8:927,1982). GCPs were then lysed and their membranes pelleted for analysis by SDS-polyacrylamide gel electrophoresis and fluorography of the resulting gels. Analysis of GCPs from brain regions devoid of optic fibers show that optic axons contribute at least 70% of labeled GCP polypeptides. The labeling of the fetal optic tract was also monitored by light microscopic radioautography of the brains. An analogous protocol was used in the adult case: Several adult eyes were each injected with ~500 µCi of ^{35}S-methionine in a volume of 1 µL. The rats were allowed to survive for 12 hours, after which the lateral geniculate nuclei and superior colliculi were bilaterally removed, mixed with unlabeled adult cortex and fractionated to produce synaptosomes, according to standard techniques. Frontal cortex from the same animals was fractionated separately as a control. Synaptosomes were lysed and the membranes pelleted for analysis. Comparison of the labeled polypeptides of synaptosomes vs. GCPs reveals major differences: There are several bands that are unique to GCPs, the major ones migrating at about 36 kd (doublet), 39 kd, and 42 kd. Conversely, there are several bands present in the synaptosome preparation but not in GCPs; The major bands have apparent molecular weights of 28 kd, 63-64 kd and 102 kd. According to widely accepted concepts, most rapidly transported proteins are associated with membranes at their destination. In conclusion, our analysis demonstrates in biochemical terms that target cell contact of the growth cone leads to the expression of a dramatically different set of polypeptides in the nerve terminal. These changes are likely to be related to membrane function. [Support: NIH NS 13466 (KHP) and a predoctoral fellowship from NSF (PS).]

342.3 NEURONAL DIFFERENTIATION ANTIGENS EXPRESSED DURING SPROUTING AND SYNAPTOGENESIS. I. Wallis*, L. Ellis and K.H. Pfenninger. Dept. of Anat. & Cell Biology, Columbia Univ. P&S, New York, NY 10032.

Monoclonal antibodies (MCAs) were generated to antigens present on growing or synapsing neurons. Synaptosomes (SSs) from adult rat cortex or growth cone particles (GCPs) from fetal rat forebrain (Ellis et al., Neurosci. Abst., 1982) were used as immunogens in the mouse. Following cloning, hybridomas were differentially screened against SSs and GCPs, using two different enzyme-immunosorption assays (EIAs). This procedure has resulted in the identification of 60 hybridomas secreting MCAs that recognize antigens on SSs or GCPs or both. Two of the better characterized MCAs are described below.

MCA 5B4-4, generated against GCPs, binds to GCPs but not to SSs in EIAs. As seen by indirect immunofluorescence, this MCA is neuron-specific, at least within the nervous system. It recognizes the antigen only in permeabilized neurons, apparently in a subplasmalemmal location. In 13-day fetal spinal cord, immature dividing neurons are not stained whereas the developing anterior horn region, spinal roots and marginal layer are strongly fluorescent. Immunoblots of GCPs have revealed binding of this MCA to a region between 230 and 250 kd.

MCA 2, generated to SSs, is SS-positive but GCP-negative in EIAs. Immunocytochemistry localized this neuron-specific antigen to the plasmalemmal surface of presynaptic terminals. More proximal regions of the mature neuron are probably also stained. The expression of this antigen is developmentally regulated: In rat cortex it first appears on about fetal day 16 and increases from then on to reach maximal levels in the adult. The antigen is sensitive to protease and denaturing agents. In summary, we have isolated MCAs that recognize neuronal molecules expressed at specific stages of differentiation, i.e., sprouting and synaptogenesis. [Support: NIH NS 13466 (KHP), NY State Health Res. Council (IW), NIH NRSA (LE)].

342.4 MODULATION OF MOTONEURON GLUTAMATE SENSITIVITY BY INTERNEURONS IN VITRO. R.J. O'Brien* & G.D. Fischbach. Dept. of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Using previously described techniques (O'Brien et al., Soc. Neurosci. Abstr., 1982, p. 129; Okun & McPheeters, Soc. Neurosci. Abstr., 1980, p. 733) we have employed fluorescein conjugates of Wheat Germ Agglutinin and Cholera Toxin to identify motoneurons amidst heterogeneous cultures of chick spinal cord. These tracers have also enabled us to use a FACS IV to sort the labeled cells and obtain cultures of motoneurons that are more than 90% pure. Using patch clamp recording techniques we found that motoneurons in sorted and unsorted cultures displayed similar electrophysiologic and morphologic properties.

Motoneurons in sorted and unsorted cultures respond to glutamate, a proposed excitatory neurotransmitter, with an ED_{50} of 87 µm and a Hill coefficient of 1.08. The response reversed at -8 mV and showed little desensitization.

By comparing the characteristics of the glutamate response in sorted versus unsorted motoneurons we obtained evidence that the level of glutamate sensitivity in motoneurons is influenced by interneurons, and that the glutamate response duration is modulated by glia. The presence of presynaptic neurons induced a 5-fold increase in the glutamate sensitivity of motoneurons, while glia decreased the duration of the response of motoneurons to a pressure application of glutamate by 3-fold.

Incubation of cultures of sorted or unsorted motoneurons with the glutamate inhibitor γ-D-glutamylglycine (GGG) for 3 days resulted in a doubling of the motoneuron glutamate sensitivity but had no effect on GABA sensitivity. Although GGG increased the glutamate sensitivity of both sorted and unsorted motoneurons, the motoneurons in unsorted cultures still retained their increased sensitivity relative to sorted motoneurons.

We conclude from these experiments that the sensitivity of motoneurons to glutamate can be regulated by the presence of presynaptic neurons and also by the level of glutamate in the media. Because the effects are additive, these mechanisms appear to be independent.

These cultures are also proving useful for studying synapses formed on motoneurons. Spontaneous EPSP's recorded from motoneurons reversed at -7 mV similar to glutamate induced currents. Synaptic potentials evoked by stimulating a presynaptic neuron as early as D3 in vitro showed multiple levels and a large number of failures.

342.5 RELEASE OF ACETYLCHOLINE FROM GROWTH CONES DETECTED WITH PATCHES OF ACETYLCHOLINE RECEPTOR-RICH MEMBRANE. R.I. Hume, L.W. Role & G.D. Fischbach. Dept. of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Studies of developing neuromuscular junctions both in vivo and in vitro have shown that synaptic tranmission can be detected within minutes after a cholinergic growth cone contacts a target muscle cell. This might mean that the ability to release acetylcholine is rapidly induced during synapse formation. An alternative possibility is that neurons are capable of releasing transmitter even before they reach their target. To examine the development of the transmitter release process, we have used excised patches of acetylcholine receptor-rich membrane as sensitive detectors of acetylcholine.

Outside-out patches were formed from cultured chick myotubes which were grown on small chips of collagen-coated cover slips. We examined release from chick ciliary ganglion neurons that had been dissociated and cultured for about 18 hours in the absence of target myotubes. At the beginning of each experiment a single glass chip with muscle cells was placed in the dish as a source of patches. To test the sensitivity of isolated patches, acetylcholine was applied from an iontophoretic pipette. Judging from the single channel current and the peak current produced by large doses of acetylcholine, the patches contained as many as 300 receptors. The amount of acetylcholine that mimicked small synaptic potentials in intact myotubes produced channel openings in isolated patches. Thus, patch electrodes can probably detect an amount of acetylcholine equal to, or less than, one quantum.

Patch electrodes were positioned within a few microns of active growth cones, and extracellular stimulating electrodes were placed on the cell bodies. When neurons were stimulated repetitively to produce a short train of action potentials, discrete channel openings were detected at about 1/3 of the growth cones that we tested (59/188). We assume that the evoked channel openings reflect acetylcholine release because their amplitude and duration were indistinguishable from that produced by acetylcholine, and because they were not observed in the presence of curare. The first openings after the onset of stimulation ranged between 40 msec and 4 sec. Our data indicate that the longer latencies cannot be accounted for by diffusion of acetylcholine from a distant source. Rather we conclude that the release occurs in the immediate vicinity of the growth cone, and that the delay is a property of an immature release mechanism.

These results show that neurons are capable of releasing their transmitter even when synaptic partners are not present. Transmitter release capability apparently can precede synapse formation.

342.6 TRANSMITTER RELEASE & PRESYNAPTIC SPECIALIZATION AT NERVE-MUSCLE CONTACTS IN VITRO. L. Role, D. Roufa* and G. D. Fischbach. Dept. Anat. & Neurobiol., Washington Univ. Sch. Med., St. Louis, MO.

Dissociated ciliary ganglion neurons (CGNs) rapidly form synaptic connections with co-cultured myotubes and induce multiple acetylcholine receptor (AChR) clusters along the length of neurite-muscle contact. We have now examined neurite-associated receptor patches (NARPs) both electrophysiologically and morphologically to determine if these contacts are sites of pre- as well as postsynaptic specialization.

To see if NARPs were sites of transmitter release, we used extracellular synaptic current recording and visualized the NARPs with two immunological markers for AChRs that do not block synaptic function. AChR patches were labeled with either (a) a globulin fraction of myasthenic serum or (b) a monoclonal antibody to Torpedo AChR (MAB 35; gift of J. Lindstrom) and visualized with a fluorescein coupled second antibody. Extracellular recording revealed synaptic currents at 59% (n=17) of the nerve associated receptor patches.

We have also examined NARP's and identified release sites by scanning EM (SEM). All but one NARP relocated in SEM had distinct specializations (n=16); at ~50% the neurite appeared flattened and embedded in the muscle surface. The remainder had numerous filipodia and webbing of the neurites. All identified release sites are of the latter category. It is possible that the "flattened" type NARPs correspond to the NARPs at which no release was detected. We plan to examine the formation of these multiple synaptic contacts and to determine if release also occurs at non-NARP areas using AChR patch-electrodes as ACh release detectors (see Hume, Role, & Fischbach; this vol.)

CGNs grown with muscle, though initially multipolar pare down to an essentially unipolar geometry (characteristic of adult CGNs in vivo) by ~1 wk in vitro. Patch clamp whole cell recording reveals that this change in geometry occurs with no concomitant changes in the electrical properties except the expected change in R_{IN} and passive charging curve. The conversion from a multipolar to unipolar geometry does not depend on the establishment of synapses per se, since CGNs become unipolar even when grown in the absence of target myotubes (n=20). In contrast, spinal motoneurons retain their multipolar geometry in vitro (n=10). Thus the attainment of a unipolar geometry in vitro may in part be the result of an inherent program in CGNs. However, the decision as to which process is maintained may depend on their relative success in forming and maintaining synapses.

342.7 CHARACTERIZATION OF THE ACHR AGGREGATING MOLECULES IN EXTRACELLULAR MATRIX FRACTIONS FROM ELECTRIC ORGAN AND MUSCLE. Ralph M. Nitkin, Earl W. Godfrey*, Bruce G. Wallace, U.J. McMahan. Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

Molecules tightly bound to the extracellular matrix (ECM) at the neuromuscular junction (NMJ) in skeletal muscle direct several aspects of neuromuscular regeneration including the aggregation of acetylcholine receptors (AChRs) at the NMJ on regenerating myotubes. We have been studying an ECM-enriched fraction from the electric organ of Torpedo californica, a tissue with a high concentration of cholinergic synapses, which causes the AChRs on cultured myotubes to aggregate into clusters. Our aims are to purify the active molecules, learn whether they are similar to those at the NMJ, and, if so, to characterize them in detail. Here we summarize our progress.

The ECM fraction is insoluble in isotonic saline and 3% Triton; 20% of the protein is collagen, based on hydroxyproline assays. The AChR-aggregating activity is selectively extracted from the ECM fraction by high ionic strength or pH 5.5 buffers, treatments that solubilize other ECM components. Antiserum against the high salt extracts binds both to the AChR-aggregating molecules and to ECM at frog NMJs.

We have purified the AChR-aggregating factor over 1000-fold using gel filtration and ion exchange chromatography. Only a few nanograms of the purified extract are required to cause detectable AChR aggregation on cultured myotubes. Assuming a molecular weight of 60-100 kd as indicated by gel filtration, this corresponds to about 10^{-10} M protein. Analysis by SDS gel electrophoresis reveals that even fractions with the highest specific activity contain several polypeptide chains. Polypeptides were electro-eluted from regions of the gel containing the material that copurified with AChR-aggregating activity and injected into rabbits. Polypeptides from the 80 kd region of the gels produced antiserum that completely blocks the activity of the crude extracts.

We do not detect significant AChR-aggregating activity in ECM fractions prepared from Torpedo muscle, but pH 5.5 extracts contain a small amount of activity. Similar ECM fractions and soluble extracts of liver are ineffective. The muscle AChR-aggregating activity could be completely blocked and immunoprecipitated by the antiserum raised against electric organ ECM high salt extract. Thus the AChR-aggregating factor associated with ECM in Torpedo electric organ is similar to an AChR-aggregating molecule in muscle and is present in sufficient quantity to allow further purification and characterization.

This research was funded by grants from the Muscular Dystrophy Association and NIH (NS 16440 and NS 14506) and postdoctoral fellowships from MDA to R.M.N. and E.W.G.

342.8 FORMATION AND DISPERSAL OF ACETYLCHOLINE RECEPTOR CLUSTERS AT NERVE-MUSCLE JUNCTION IN XENOPUS CULTURE. H. Kuromi* and Y. Kidokoro. The Salk Institute, San Diego, CA. 92037.

At the adult vertebrate neuromuscular junction, acetylcholine receptors (AChRs) are highly localized at the junctional region. Early embryonic muscles, however, have AChRs distributed over the entire surface, and during development AChRs accumulate to the junctional region. In order to clarify the mechanism by which nerve induces acetylcholine receptor localization at its contact sites on the muscle cell membrane, we investigated the formation of receptor clusters at nerve-muscle junction and their disappearance after denervation by observing the sequential changes in AChR cluster distribution on Xenopus cultured muscle cells with image-intensified microscopy. AChRs on the muscle cells were labelled with rhodamine-conjugated α-bungarotoxin (R-αBT). During nerve-induced AChR accumulation, initially small AChR clusters (less than 1 μm in diameter) emerged at nerve-muscle contact sites from the background. They grew in size, increased in number and fused to form larger clusters. The rate of individual AChR cluster formation varied from 27 binding sites/hr to 1980 binding sites/hr assuming the density of αBT binding sites within the cluster as 900/μm^2. We estimated a diffusion coefficient for diffusely distributed AChRs using the fluorescence photobleaching recovery method as 1.45×10^{-10} $cm^2 \cdot sec^{-1}$ at 22°C. Diffusion of AChRs in the muscle membrane can account for the rate of AChR cluster formation at nerve-muscle junction according to the simple passive diffusion-trap mechanism (Edwards and Frisch, 1976). If the trap size were fixed, it is conceivable that the rate of receptor cluster formation decreases as the trap is filled with receptors. Unexpectedly, it was found that the rate increased with receptor cluster size. This evidence may be explained by a hypothesis that the trap size is not fixed and increases during development. When the nerve causing receptor accumulation was severed by a strong focussed laser light, the receptor clusters along the nerve dissipated. The rate of receptor cluster dispersal after denervation was much slower than that predicted by the diffusion trap model, suggesting that once receptors were trapped in a cluster at nerve-muscle contact region, the diffusion of receptors was slowed even when the nerve was lost. Furthermore, the majority of receptor clusters at nerve-muscle junction disappeared at 7 hrs after denervation in 1-day innervated muscle cells, while about 50% of receptor clusters remained in 3-day innervated muscle cells. This result suggests that the stabilization of receptor clusters occurs at nerve-muscle junction during days in culture.

342.9 DENERVATION BLOCKS THE NORMAL POSTNATAL DECREASE IN RAT ENDPLATE CHANNEL OPEN TIME. S.M. Schuetze and S. Vicini*. Dept. of Biological Sciences, Columbia Univ., New York, N.Y. 10027

Developmental interactions between nerve and muscle cells have been of great interest in recent years. One developmental change in muscle that apparently results from innervation is a three-fold decrease in the mean open time of ACh-sensitive channels. In newborn rats, the mean channel open time (τ) of endplate acetylcholine receptors (AChR's) is about 4.5 msec at 21°C, compared to about 1.5 msec in adults (G. Fischbach & S. Schuetze, J. Physiol. 303: 125, 1980). In rat soleus muscles, the switch occurs between postnatal days 8-18 as channels with long open times gradually disappear while channels with short open times increase in number. We have followed the fate of channels in endplates that were denervated shortly after birth, before τ began to shorten, to determine if continual innervation is required for the decrease in τ. We have found that denervation blocks, or at least delays, the shortening of τ.

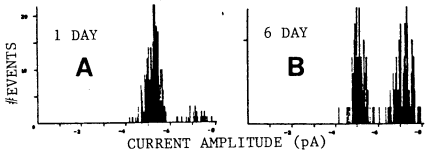
The lower left hindlimb of neonatal rats was denervated by removing a length of the sciatic nerve. Several days later, the denervated soleus muscle was removed and stained for acetylcholinesterase (AChE) activity. The AChE stain marked the vacated endplate sites, which could not readily be located physiologically. The mean channel open time of AChR's was determined by fluctuation analysis of focal extracellular recordings of ACh-induced membrane currents (S. Schuetze, J. Physiol. 303: 111, 1980). We measured τ at 28 denervated endplates in 7 different soleus muscles from rats ranging in age from 8-18 days. In all cases, τ at the denervated endplates was long, that is, similar to τ's found in newborn rat endplates. In contrast, τ at the contralateral innervated endplates decreased three-fold with age, as in normal muscles. The long open times at the denervated endplates did not result from the AChE staining, for τ at innervated endplates was unchanged by staining.

Unexpectedly, we found enhanced extrajunctional ACh sensitivity on either side of most endplates, for several hundred micrometers, in the contralateral innervated muscles of the denervated rats. This observation allowed us to compare the open times of channels in junctional and extrajunctional regions of the same fibers. We found that τ in these extrajunctional regions decreased in parallel with τ at the endplates. Thus, innervation effects a developmental decrease in τ not only at the endplate, but also in nearby extrajunctional regions (cf. Kullberg et al., Nature 289: 411, 1981).

342.10 DEVELOPMENTAL ALTERATIONS IN ACETYLCHOLINE RECEPTOR CHANNEL PROPERTIES PROCEED IN THE ABSENCE OF INNERVATION. P. Brehm, F. Moody-Corbett, and Y. Kidokoro†. Tufts Univ. School of Medicine, Boston, MA 02111, and †The Salk Institute, San Diego, CA 92138.

Noise analysis studies of ACh receptor channels on Xenopus myotomal muscle have indicated that alterations in the ACh receptor channel kinetics occur during development in vivo[1] and that these kinetic changes can occur on muscle cells grown in cell culture[2]. In the present study single channel recording of ACh activated channels allowed us to examine both conductance (γ) and kinetics of ACh receptor channels on developing Xenopus muscle in culture. Muscle cells were dissociated prior to nerve contact (Stg 17) and cultured in the absence of nerve. Recordings were made in a solution containing 120 Na, 1.6 K, 1 Ca, 8 HEPES (mM) at room temperature. Two amplitude classes of ACh activated channels were seen having slope γ of 60 ± 10 pS (n = 12) and 42 ± 8 pS (n = 12) over the range of hyperpolarized potentials from 0 to 60 mV. When recordings were made within the first 24 hours in culture the lower γ channel comprised a high proportion (92 ± 9%) of the total number of events (A) after correction for unresolved openings. By contrast, in 3 to 6 day cultures the lower γ channel comprised a smaller proportion (37 ± 20%) of the total number of events recorded (B). These two γ classes could also be distinguished on the basis of kinetic differences. The apparent mean channel open time from single channel analysis was 2.2 ± 0.7 msec (n = 10) for the lower γ channel and 0.85 ± 0.2 msec (n = 6) for the higher γ channel at resting membrane potential. A switch in the relative activity of these two channel types during development could, therefore, account for the change in overall ACh receptor kinetics which has been observed with noise analysis[1,2]. The results of the present study support the view that an age dependent increase in the expression of a large γ channel with fast kinetics occurs on developing muscle. Furthermore, the observation that a switch to the adult form of the ACh receptor occurs in muscle which has never directly received nerve contact indicates that altered receptor expression during development can occur in the absence of nerve. Supported by NIH, MDA and MDAC.

1. Kullberg et al. (1981) Nature 289, 411.
2. Brehm et al. (1982) Develop. Biol. 91, 93.



342.11 SINGLE CHANNEL OPEN TIMES OF TWO TYPES OF ACh RECEPTORS IN CULTURED XENOPUS MYOCYTES DECREASE DURING DEVELOPMENT. R.J. Leonard*, Y. Nakajima, S. Nakajima, and T. Takahashi*. Dept. of Biological Sciences, Purdue University, West Lafayette, IN 47907.

Myotomes from stage 15-17 *Xenopus* embryos were dissociated and grown in culture (60% L-15, 1% fetal calf serum, with or without penicillin/streptomycin) in the presumed absence of any neural influence. Single channel currents evoked by 500 nM ACh were measured using the 'giga-seal' patch clamp technique of Neher and Sakmann.

In *Xenopus* myocytes the first emergence of ACh receptors (AChRs) and the development of sensitivity to ACh is known to occur at about stage 20 (1,2). Recordings obtained from as early as stage 22/23, at which all of the receptors are newly-inserted, show the coexistence of two classes of AChRs; one with a large conductance and short open time (large-g) and another with a smaller conductance and longer open time (small-g) as described previously for more mature cultures (3,4).

The conductance values of the large-g and small-g AChRs remained constant throughout the course of development from stage 22 up to 6 days in culture (stage 48). In contrast, the mean open times of both AChR types were long at stages 22-26, and became shorter as development proceeded *in vitro*. The degree of developmental change was greater for the small-g AChRs; however, at all stages, the open time of the large-g class was the shorter of the two. Open times of both classes are strongly voltage and temperature dependent. At 50 mV hyperpolarized from rest and 13°C, the mean open times at stage 22/23 were 6.5 msec for the large-g type and 30.0 msec for the small-g type, while the corresponding values at stage 48 were 4.4 msec and 7.2 msec, respectively. There was no apparent change in the estimated resting potential of the myocytes over the time-course of these experiments.

These results indicate that the shortening of mean channel open time previously measured *in vivo* (5) and *in vitro* (6) using noise-analysis is not due solely to a redistribution in the relative numbers of the two types of AChRs on the membrane, but also to a change in the individual characteristics of each receptor type. It remains to be seen whether the change in open times is a result of modification of the receptors themselves, or rather to some change in the membrane environment.

Supported by NS08601 and T32-GM-07211.

1. Blackshaw and Warner (1976) Nature 262:217-218.
2. Bridgeman et al. (1981) Soc. Neurosci. Abstr. 7:702.
3. Clark and Adams (1981) Soc. Neurosci. Abstr. 7:838.
4. Greenberg et al. (1982) Biophysical J. Abstr. 37:18a.
5. Kullberg et al. (1981) Nature 289:411-413.
6. Brehm et al. (1982) Dev. Biol. 91:93-102.

342.12 ON THE TIME COURSE OF THE FORMATION AND THE ELIMINATION OF ACH RECEPTOR CLUSTERS. H. Benjamin Peng* (SPON: C. Anderson). Dept. of Anatomy, Univ. of Illinois, College of Medicine, Chicago, IL 60612.

In cultures of Xenopus myotomal muscle cells, the formation of acetylcholine receptor (AChR) clusters can be induced by positive polypeptide-coated latex beads (J. Neurosci. 2:1760). In this study, we have examined the development of new AChR clusters and the diappearance of the existent clusters induced by the beads.

AChR clustering was initiated by applying 4.5 μm polyornithine-coated latex beads to Xenopus muscle cells cultured on coverglass. The clusters were assayed by tetramethylrhodamine-conjugated α-bungarotoxin labeling followed by fluorescence microscopy. The mean percentage of bead-muscle contacts associated with AChR clusters was used as an index.

After an overnight bead-muscle coculture, AChR clusters could be detected at a saturation level of 60-70% of the bead-muscle contacts. Prolonged incubation did not increase this value. In 1hr cocultures, clusters were detected at 30% of the final level. At 2 hrs, the clustering already reached 40 to 70% of the final level and, between 4 to 8 hrs, it reached 90%. The bead-induced clusters observed in cocultures younger than 4 hrs were small and dim as compared with overnight clusters. Nevertheless they could be clearly visualized and photographed with conventional fluorescence optics. Subsequently, both the size and the fluorescence intensity of the clusters increased with time.

In the absence of bead treatment, a class of large (over 10 μm long) AChR clusters was observed on the substrate side of over 50% of the muscle cells. As new clusters were formed at the bead-muscle contacts, we observed that these large clusters disappeared with time. However, this process took a much longer time course than the formation of new clusters. Whereas in 1-day cocultures, these large clusters could be detected in 50% of the bead-contacted cells, only 10% of these cells exhibited such clusters after 2 days.

These studies indicate that AChR clusters can form rapidly (within 1 hr) in response to polypeptide-coated latex beads in cultured Xenopus muscle cells. They rule out the possibility that the beads merely become associated with existent clusters. In addition, these beads also cause a dispersal of the existent clusters with a longer time course. Together, these two properties seem to bear striking resemblance to the effects of innervation. (Supported by NIH grant NS 16259 and MDA)

343.1 IMMUNOELECTRONMICROSCOPY OF NEURAL ANTIGENS ON ULTRATHIN FROZEN SECTIONS. M.R. Celio[1], G. Keller[2], and F.E. Bloom[1]. (SPON: J.H. Steinbach). [1]A.V. Davis Center for Behavioral Neurobiology, Salk Institute, P.O. Box 85800, San Diego, CA 92138 and [2]Dept. Biology, UCSD, San Diego, CA 92093.

The ultrastructural localization of neural antigens generally relies on immunoperoxidase staining of thick vibratome sections (preembedding staining), subsequent embedding in resin and ultrathin sectioning. This procedure may be accompanied by diffusion artifacts which render subcellular localizations inaccurate. An alternative way of localizing antigens is to incubate ultrathin sections of plastic embedded tissue with the antibody (post embedding staining); in this case however, the harsh treatment of the tissue damages and distorts many antigens making their detection impossible. We have employed a third alternative: immunoferritin (or immunogold) staining of ultrathin frozen sections (non embedding staining) according to Tokuyasu (Histochem. J. 12, 381-403, 1982) and examined its suitability in the ultrastructural detection of proteins and peptides in the nervous system. By this method we have detected protein I (a regulatory protein) and parvalbumin (a calcium binding protein) in the cerebellum; somatostatin (a neuropeptide) in the substantia gelatinosa of the spinal cord and tubulin (a structural protein) in peripheral nerves. Protein I was found associated with the cytoplasmic face of the synaptic vesicle membrane. No labeling of the presynaptic membrane or of the postsynaptic density was noticed. Parvalbumin is distributed all over the cytoplasm, without a preferential binding to any recognizable cell organelles or to the postsynaptic density. This observation diverges clearly from preembedding staining, in which an association of anti-parvalbumin label with all membranes was observed. Somatostatin is found exclusively in large, granular vesicles of presynaptic terminals. This result is similar to that obtained with Substance P antisera and supports the hypothesis that the large granular vesicles are the storage site of peptides. Alpha-tubulin antibodies decorate arrays of microtubules in the axoplasm of peripheral nerves. These preliminary observations clearly indicate the usefulness of ultracryomicrotomy for the ultrastructural localization of neural antigens. In 3 out of the 4 studied antigens we gained additional information not available with other techniques. Immunolabeling on ultrathin frozen sections is very reproducible, displays a high intensity and specificity of staining and good preservation of ultrastructural details. (Supported in part by the Swiss National Foundation.)

343.2 MYELIN ASSOCIATED GLYCOPROTEIN (MAG) IN MÜLLER CELLS OF HUMAN RETINA. K. Stefansson[+], M.L. Molnar[+], L.S. Marton[+], G.K. Molnar[+], M. Mihovilovic[+], and D.P. Richman[+]. (SPON: B.G.W. Arnason). +Department of Neurology and The Brain Research Institute, University of Chicago, Chicago, Illinois 60637*.

Rat monoclonal antibodies ($BRIC_{37}$) were raised against MAG. On Western blots $BRIC_{37}$ binds to isolated human MAG and on Western blots of homogenates of human CNS tissue it binds only to antigens with a relative mobility identical to that of MAG. $BRIC_{37}$ identifies nothing on Western blots of human liver, kidney or striated muscle. Immunohistochemical distribution of the antigen with which $BRIC_{37}$ reacts is identical to that described for MAG (Sternberger et al Natl Acad Sci, USA 76, 1510, 1979). The presence of MAG in human retina was demonstrated immunohistochemically, using $BRIC_{37}$ and serum from a patient with monoclonal gammopathy and neuropathy whose serum monoclonal antibody reacts with human MAG (Stefansson et al Acta Neuropath 59, 255, 1983). The retina is to date the only unmyelinated part of the nervous system that has been shown to contain MAG. On Western blots of homogenates of human retina the antibodies recognized antigens with relative mobilities compatible with MAG. In the retina MAG is confined to the surface of Müller cells which are considered to bear a strong resemblance to astrocytes of the brain. However, in the nerve fiber layer of chicken retina the Müller cells form a myelin-like sheath around thick axons (Inoue et al Okajimas Folia Anat, Jan 57, 79, 1980) and must, therefore, be considered to be somewhat related to oligodendrocytes and Schwann cells. The presence of MAG on Müller cells in human retina underscores that relationship.

343.3 A 2':3'-CYCLIC NUCLEOTIDE SYSTEM IS ASSOCIATED WITH THE VERTEBRATE PHOTORECEPTOR. D. J. Giulian (SPON: W. Strittmatter). Program of Neuroscience, Baylor College of Medicine, Houston, Texas 77030

Though the enzyme, 2':3'-cyclic nucleotide 3'-phosphohydrolase (2':3'-cNMP), has long been associated with oligodendroglia of the CNS, the natural substrate and function of the 2':3'-cyclic nucleotide system remains unknown. More recently, 2':3'-cNMP activity has been identified within the vertebrate retina (Giulian, Brain Res. 189:135-155, 1980). In an attempt to determine the cellular origin of the retinal 2':3'-cNMP, a new histochemical staining technique was developed which links the hydrolysis of 2':3'-cNADP to the formation of a reduced insoluble tetrazolium formazen. Photoreceptors from bovine, rat, and fish retina are stained by this procedure. Polyclonal antibodies prepared against 2':3'-cNMP from bovine brain are found to cross react with bovine and rat retinal enzymes. Peroxidase-labeled antibody shows by electron microscopy that the enzyme is located along the plasma membrane of the inner segment of photoreceptors. The association of 2':3'-cNMP with the photoreceptor suggests a role for 2':3'-cyclic nucleotides in visual function and presents an opportunity to identify the enzyme's natural substrate.

343.4 VIP-POSITIVE CELLS IN THE RAT VISUAL CORTEX. Alan Peters and James R. Connor. Dept. of Anatomy, Boston Univ. Sch. of Med. Boston MA 02118

The role of bipolar neurons in cortical circuitry has been a question of interest to this laboratory for some time. Feldman and Peters (J.Comp. Neurol.,1978) describe the Golgi impregnated appearance of these neurons as being characterized by a fusiform cell body with vertically oriented dendrites generally arising from two primary dendrites at opposite poles of the cell body. A Gogli-EM study (Peters and Kimerer, J.Neurocytol.,1981) further describes bipolar neurons as having nuclei with a vertically oriented cleft. The cells have few axosomatic synapses, some of which are symmetric and others asymmetric. The axons of the bipolar neurons studied with the Golgi-EM technique all form asymmetric synapses and most of them occur on dendritic spines.

It has been suggested by a number of studies that antibodies to vasoactive intestinal polypeptide (VIP) react with a population of neurons in the cortex,many of which are bipolar neurons. Therefore, we utilizied anti-VIP to further study bipolar neurons. The light microscopic portion of this study reveals the highest concentration of VIP-positive neurons in layers II-III. There are fewer cells in layer V, and theleast number are found in layer IV. As in Golgi impregnation studies, the VIP-positive bipolar neurons have fusiform cell bodies and primary dendrites which emanate from opposite poles of the cell body to form a narrow, vertically oriented dendritic tree. The descending dendrite of these cells will frequently splay into thin, beaded branches which are unremarkable at the electron microscope level. The nuclei of the VIP-positive bipolar neurons generally have a vertically oriented cleft, and there are few axosomatic synapses. Those axosomatic synapses present are both symmetric and asymmetric.

The VIP-positive axons present in the preparation are different from those of the Golgi impregnated neurons. The VIP-positive axons do not synapse with dendritic spines. Instead, they synapse preferentially with the shafts of small diameter dendrites and the synapses they form are more symmetric in type. That is, they lack an obvious postsynaptic density but have a wider synaptic cleft than is typical of symmetric synapses.

Explanation of these data is difficult for we are apparently faced with two populations of neurons that are similar in appearance at the light microscopic level, but different in their synaptic connections.

Supported by N.I.H. Grant No. NS 07016 and N.I.A. Grant No. AG 00059

343.5 LUTEINIZING HORMONE RELEASING HORMONE (LHRH) SYNAPSES IN THE DIAGONAL BAND (DBB) AND PREOPTIC AREA (POA) OF THE GUINEA PIG. A.J. Silverman, Dept. Anatomy and Cell Biology, Columbia Univ., P&S, NY, 10032.

Immunocytochemical procedures were developed that resulted in excellent ultrastructural preservation of tissue and in the demonstration of numerous LHRH containing synapses in the DBB and POA. Adult male guinea pigs were perfused via the heart with 500 ml of fixative containing 0.075M lysine, 0.01 M sodium periodate and 4% paraformaldehyde in 0.1M phosphate buffer, adjusted to pH 7.0. Following perfusion tissue was fixed for an additional two hours by immersion; sections were then cut at 50 um on a vibratome. Sections were washed overnight in buffer at 4 C and then washed again on the following day in 0.1M sodium periodate (10 min) and in 0.1% sodium borohydride (10 min). Following numerous buffer washes, sections were transferred to primary antiserum (LR-1,Benoit) diluted with 0.02% saponin for 48 hrs, 4 C. Sections were washed and the second (biotinylated goat anti rabbit IgG) and third (avidin-biotin-HRP) layers were applied for 60 min each. HRP was demonstrated by incubation in a DAB solution containing 50 mg DAB, 200 mg glucose, 40 mg NH_4Cl and 0.3 mg glucose oxidase per 100 ml of buffer. With the glucose oxidase slowly generating the H_2O_2, very small amounts of HRP could be visualized by continuing the incubation for 1-3 hrs. All of these reactions were done at room temp. Cytoarchitectonic regions are then dissected, tissue post-fixed in 2% OsO_4 with 1.5% potassium ferricyanide for one hr and embedded in Epon. The beaded axonal processes seen in the light microscope in the DBB and POA could be located in the thin sections. LHRH axons traveled in bundles with small, unmyelinated non-reactive axons and were frequently the largest axon in the bundle. Such a profile represented the intervaricose region of the axon. There were numerous instances where these processes swelled and formed en passant synapses with non-immunoreactive dendritic profiles. The LHRH positive synapses contained mitochondria, clusters of small lucent round synaptic vesicles and 100-200 nm diameter neurosecretory granules. There was a well-defined synaptic cleft. Most of these synaptic profiles were of the symmetric variety. These synapses are in the same area of the CNS as the largest number of LHRH cell bodies. These observations indicate that LHRH may well be a neurotransmitter and could exert an important synaptically-mediated influence on neighboring neurons. Supported in part by USPHS HD10665 and Whitehall Fdn.

343.6 MONOCLONAL ANTIBODIES AS PROBES OF MEMBRANE COMPARTMENTATION. R.M. Pruss*, E. Mezey*, E.A. Shepard*, and L.E. Eiden*. Laboratory of Cell Biology, NIMH, NIH, Bethesda, MD 20205.

The adrenal medullary chromaffin cell stores both neuropeptides and catecholamines in intracellular granules. The contents of these granules are released by a Ca^{++} dependent mechanism in response to depolarizing signals. In order to study membrane and organelle specialization, processing, and recycling, we have made monoclonal antibodies to chromaffin cell membrane proteins.

Two monoclonal antibodies, 7A3 and 3C1, display differential binding to subcellular membrane fractions prepared from chromaffin cells. Antibody 7A3 binds to an antigen present in chromaffin granule membranes but absent from plasma membrane, mitochondria, lysosomes and nuclei. Conversely, antibody 3C1 binds to a family of proteins excluded from chromaffin granules but present in all other chromaffin cell membrane fractions. Both the 7A3 and the 3C1 antigens are expressed in Golgi fractions of these cells. Electron microscopic examination of sections from bovine adrenal medulla confirmed that 7A3 binds to chromaffin granules and to Golgi membranes. Using the peroxidase-antiperoxidase technique, the 7A3 antigen appears to be present on the inner surface of both these organelles.

Because of their differential localization, these antigens should be useful markers for studying membrane protein synthesis, compartmentation, recycling, exchange, and degradation. Chromaffin cells from bovine adrenal medulla can be cultured and fractionated into various subcellular components. The cell culture conditions can be manipulated to alter the chromaffin granule contents. The availability of monoclonal antibodies to normally expressed membrane proteins provides a means of probing differential membrane processing in response to external stimuli.

343.7 PNMT NEURONS IN THE CENTRAL NERVOUS SYSTEM: NEW ANATOMICAL AND BIOCHEMICAL FINDINGS. D.A. Ruggiero, M.P. Meeley, C.A. Ross, D.H. Park, T.H. Joh and D.J. Reis, Lab of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

We sought to establish the distribution of neurons and processes in the central nervous system that contain the adrenaline synthesizing enzyme, phenylethanolamine N-methyltransferase (PNMT), using the PAP-technique. Adult Sprague-Dawley rats were perfused with 4% formalin and sections stained with antibodies to PNMT, tyrosine hydroxylase (TH), dopamine-B-hydroxylase (DBH), or L-amino acid decarboxylase (AADC) purified from bovine adrenal medulla. Cell body staining was enhanced in some rats by administration of colchicine (COL) (150 ug/10 ul) into the lateral ventricles. (1) Cell Bodies: In control animals, PNMT neurons were restricted to the two traditional groups (Hokfelt et al., Brain Res. 66:235,1974) in rostral ventrolateral (C1) and dorsomedial (C2) medulla. These neurons also stained for TH, DBH, and AADC. Twenty-four hours after COL, PNMT was visible in numerous hypothalamic (Hyp) perikarya within lateral, perifornical, dorsomedial and zona incerta nuclei. PNMT cell bodies in most Hyp areas did not stain for TH, DBH, or AADC. The increased PNMT staining in Hyp with COL was paralleled by an increase in PNMT activity measured in micropunch preparations of medial Hyp, from 6.33 + 1.94 to 11.4 + 3.1 pmoles, E/mg/15 min (n=6, p .005). PNMT activity also increased following COL in C1 area, but not in midline thalamus. Immunotitration demonstrated that the increase in hypothalamic PNMT was due to accumulation of specific enzyme protein. TH activity was not increased in Hyp by COL, but increased 2-3 fold in the C1 area of the medulla. (2) Terminal Fields: PNMT containing terminals were located in regions as described by Hokfelt et al. (1974) including motor vagal n., locus ceruleus, central grey, thalamic and Hyp n. New terminals were seen in additional substructures of visceral control including: n. tr. solitarius, raphe n., parabrachial n., dorsal tegmental n., new Hyp. n., amygdala and n. stria terminalis. (3) Pathways: PNMT fibers were recognized in three pathways including: (a) principle tegmental bundle (including the C1-spinal tract); (b) periventricular bundle; (c) transtegmental pathway.

We conclude: (1) the hypothalamus contains new groups of heretofore undescribed neurons expressing catalytically active PNMT;, these do not necessarily synthesize adrenaline since they lack detectable TH. (2) Projections and functions in CNS formerly attributed to PNMT cells in medulla may also receive contributions from hypothalamic PNMT cells as well. (3) PNMT cells and processes are highly concentrated within nuclei of visceral control and diffuse cortical projection. These findings support the view that central PNMT neurons may modulate autonomic functions in concert with cortical activity. (4) Neurons in the hypothalamus may produce as yet unrecognized methylated amines. (Supported by Grant HL18974).

343.8 ANTI-5-HT-LIKE ANTIBODIES AND THEIR IMMUNOREACTIVITY TO β-CARBOLINES: AN IMMUNOCYTOCHEMICAL STUDY. J. Pecci Saavedra, A. Brusco* S. Peressini*and D. Oliva*. Instituto de Biología Celular, Facultad de Medicina, Universidad de Buenos Aires, Argentina.

Since the introduction of 5-HT antibodies to immunocytochemistry by Steinbusch (Neuroscience 3, 811, 1978) a number of laboratories have applied this methodology to studies in the central and peripheral nervous system, with the light and electron-microscope. In a recent work Brusco et al. (J. Histochem. and Cytochem. 31, 524, 1983) showed the actual specificity of anti-5HT antibodies, prepared in our Laboratory according to the original protocol of Ranadive and Sehon as applied by Steinbush. In that work we showed that the specificity of the anti-5HT, antibody is for the β-carboline derivatives of 5-HT as a result of cyclization of the lateral chain. Spectrofluorometric, hemagglutination, standard inmunochemical tests as well as the Larson test were applied. These results were interpreted as a consequence of the use of formaldehyde which acted both as a fixative in the preparation of the tissue, and as the coupling agent in the preparation of the inmunogen. Following this line we have fixed several brain stem specimens with 0.5% p-benzoquinone or 3% glutaraldehyde which do not produce cyclization of 5-HT into β-carbolines, and which immobilize monoamines in situ (Pearse, A.G.E., Churchill Livingston Ed. 1980). As expected, the antibodies applied according to the PAP-Sternberger technique did not stain the neuronal bodies of the raphe system, using glutaraldehyde or p-benzoquinone. We consider these results as additional evidence for the proposed (v.s.) specificity of our antibody to β-carbolines. Further work is needed to confirm this feature of the anti-5HT "like" antibodies and to determine more exactly the nature of the antigenic determinants.

Work supported by Grants from the CONICET and SUBCYT, Argentina.

343.9 VARICOSITY PATTERNS AND RARE NEURON TYPES IN THE LEECH CENTRAL NERVOUS SYSTEM. T. Flanagan and B. Zipser (SPON: A. Berlind). Cold Spring Harbor Laboratory, Cold Spring Harbor NY 11724.

Our studies indicate that the neuropile of leech ganglia is compartmentalized with reference to the distributions of several types of immunoreactive varicosities. The leech neuropile is also compartmentalized with reference to the characteristic distribution of neurites from identified cell types. We suspect that such compartments may correspond to regions restricted to specific types of cellular communication. We report the results of our immunocytological studies of 11 varicosity patterns, and an in depth morphological and electrophysiological description of one set of these immunoreactive cell types.

Monoclonal antibodies (MAbs) raised against leech nerve cords react with specific neuronal antigens. Of 1200 hybridoma lines screened, 57% were immunoreactive, 3% stain restricted neuronal sets, and 1% stain varicosities. These immunoreactive varicosities resemble leech varicosities stained with commercial anti-leu-enkephalin antisera in terms of varicosity size, approximate number, and ganglionic distribution pattern. Varicosities extend throughout the leech nervous system, and are particularly conspicuous within the cephalic neurohemal sites. Antisera which stain varicosities also stain a restricted number of immunoreactive soma which either lie clustered within specialized ganglia or regularly distributed in sparce cell sets in segmental ganglia. The MAb LAN 3-11, for example, stains soma within the suboesophageal and the second segmental ganglia. These cells are well suited for electrophysiological and morphological analysis, and allow us to demonstrate that immunoreactive varicosities arise from immunoreactive soma. Using double-labeling methods, we have established that these cells are interganglionic interneurons projecting to ganglionic sites which contain immunoreactive varicosities, and that their neurites display swellings comparable to these varicosities. We recognize three LAN 3-11 subtypes, and thus we are presently studying the extent to which LAN 3-11 projection fields overlap and varicosity distribution patterns correspond. Perhaps distinct neuropile target sites will suggest separate follower cell types, and shed light on their individual physiological functions.

343.10 MOLECULAR SPECIFICITY OF LIMBIC SYSTEM NEURONS REVEALED BY A MONOCLONAL ANTIBODY. P. Levitt. Dept. Anatomy, The Med. Coll. of Pennsylvania, Phila., PA 19129.

The mechanisms that underlie the formation of systems during neural development are still a matter of conjecture. One possibility is the presence of specific chemical markers on related cells. We have used the monoclonal antibody technique to generate sets of unique markers for cells in the mammalian central nervous system. One monoclonal antibody that we produced reveals a unique distribution of a cell surface antigenic determinant restricted almost entirely to neurons of the limbic system.

A crude membrane fraction was prepared from hippocampus of adult albino rats and injected into mice. Hybridomas were produced by conventional fusion techniques and screened for specific antibody production by immunofluorescence. Clones eliciting positive staining in the hippocampus were expanded and subcloned. Hybridoma clone 2G9 produced an antibody of the IgG2a subclass that revealed a unique and specific staining pattern in the adult rat brain. Immunofluorescence and immunoperoxidase staining was analyzed along the entire neuraxis. The monoclonal antibody recognizes an antigenic determinant found almost exclusively on neurons in cerebral cortex and subcortical nuclei considered to comprise the limbic system. Only the superficial layers of the superior colliculus and cerebellar molecular layer are exceptions. In cerebral cortex, dense staining around cell bodies and dendrites is present only in medial prefrontal, sulcal, cingulate, entorhinal and subicular regions. Pyramidal and granule cells in the hippocampus and neurons in the amygdala are also strongly immunoreactive. In the basal forebrain, the septal nuclei, nucleus accumbens (including dorsomedial caudate) and bed nuclei of the stria terminalis stain with the antibody. Olfactory bulb, olfactory tubercle and piriform cortex do not stain. Most nuclei of the hypothalamus contain stained neurons but with varying density. In the thalamus, only the anterior, mediodorsal, paraventricular and lateral dorsal nuclei stain. The primary sensory and motor thalamic nuclei display no reactivity. All the brainstem nuclei that receive a descending limbic input are immunopositive, leaving almost all other regions unstained. The immunoreactive areas include the parabrachial nuclei, locus coeruleus, nucleus of the solitary tract and dorsal motor nucleus of the vagus. In the spinal cord, only the autonomic preganglionic nuclei and lamina II of the dorsal horn stain. The distribution of this antigenic determinant almost exclusively to limbic system neurons fosters the concept of molecular specificity among functionally related neurons.

Supported by NIH grant NS19606, MOD Basil O'Connor Starter Grant 5-348. P. L. is a Sloan Foundation Fellow, and by the Office of Mental Health of the Commonwealth of Pennsylvania.

343.11 ANALYSIS OF A NEURON SPECIFIC LEECH ANTIGEN. B. Zipser, T. Flanagan*, E. Macagno and R. Stewart. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724.

Is there a common denominator to leech neurons carrying the Laz2-1 antigen? In the CNS, the monoclonal antibody labels about 40 odd neurons, or 20 bilateral pairs of different neurons. Five of these neurons have been identified through intracellular dye injections and all of them discovered to project their primary axons into roots enabling them to innervate the periphery as sensory or motor neurons. Among these neurons are both pairs of the extensively studied primary mechanosensory neurons responding to pressure. The other three cells are of yet undetermined function. Of particular interest is the one pair next to the Retzius cells that is the most likely candidate to be the first neuron expressing the Laz2-1 antigen during CNS development. The expression of the Laz2-1 antigen has been extensively characterized both embryonically and post-embryonically and seen to appear in a precise spatial and temporal pattern both within the central nervous system and in the periphery.

In the periphery Laz2-1 stains putative sensory neurons in each annulus of the bodywall. It also stains neurons within the esophagus and cells that appear to be part of the testicular primordia. A possible mechano- or chemosensory function of the peripheral neurons remains to be explored. It is of considerable interest to us to identify the functions of the other Laz2-1 stained CNS neurons to determine whether they, like the pressure cells, can be tied in with first or higher order sensory function in our attempt to find a common functional denominator for Laz2-1 labeled neurons.

Our other interest in the Laz2-1 pattern relates to the temporal pattern of antigen expression in the CNS. Measured immunocytochemically, some of Laz2-1 labeled neurons only express their antigen post-embryonically. This offers the opportunity to analyze a given neuron before and after antigen expression has begun. Since some of the neurons within the staining patterns are known to be synaptically connected the possibility arises that neurons accumulate the Laz2-1 antigen as they become sequentially inserted in a functional network.

343.12 THE MACROGLIAL CELLS OF THE LEECH ARE MOLECULARLY HETEROGENEOUS. M.S. Flaster and B. Zipser. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724.

Using monoclonal antibodies (mABs) obtained from either whole nerve cords or CNS extract run on polyacrylamide gels of the leech Haemopis marmorata, we've catalogued the distribution of several mABs directed against the macroglia of the nerve cord and partially characterized some of these glial antigens biochemically. The identifiable macroglia of the leech, those in the lateral connectives, the ganglionic neuropil, the ganglionic packets and the ganglionic roots are distinguishable anatomically by postion. Here, we report several mABs which prominently stain some macroglia, but not others, differentiating these cells at the molecular level. One mAB stains the processes of the macroglial cells of the lateral connectives, the ganglionic neuropil and the ganglionic roots but does not stain the packet glia appreciably. Immunoblots of SDS gels indicate that this mAB binds a single protein antigen with an apparent molecular weight of 130 kD. The antigen is a glycoprotein. A second mAB strongly stains throughout the interior of the connective and root macroglia but does not appear to stain the other macroglial cells, while a third mAB stains throughout the interior of only the connective macroglial cells (Zipser, B. and McKay, R., Nature 289:549, 1981; and Hockfield, S. and McKay, J. Neurosci 3:369, 1983). This mAB binds a single band of apparent molecular weight 77 kD and is not glycoprotein as judged by multiple lectin column chromatography. The underlying significance of the differences in these identified glia to the development or adult function of the leech nervous system remains to be thoroughly explored although in developmental studies, the time of appearance of one of these mABs has already been established (Macagno, E.R., Stewart, R. and Zipser, B., J. Neurosci, in press).

344.1 MASS-FRAGMENTOGRAPHIC IDENTIFICATION OF THE EXCITOTOXIN QUINOLINIC ACID: ONTOGENETIC CHANGES AND MODULATION OF ITS CONCENTRATION IN THE RAT BRAIN BY TRYPTOPHAN AND BY P-CHLOROPHENYLALANINE.
G. Lombardi*, R. Corradetti*, V. Carlà*, C. Aldinio* and F. Moroni. Dept. of Pharmacology, University of Florence, 50134 Florence, Italy and Dept. of Biochemistry, Fidia Res. Lab., Abano Terme, Italy.

2,3 Pyridine-dicarboxylic acid (Quinolinic acid, QA) is an endogenous metabolite that produces axon sparing lesions in the rat brain (Schwarcz et al. Science 219, 316, 1983). Its presence in the mammalian brain has not been previously demonstrated. We developed a mass-fragmentographic method, based on the separation of the molecule with Dowex columns and esterification of its carboxylic groups with hexafluoroisopropanol, that is capable of measuring QA in approximately 50 mg of brain tissue. Using this method QA has been identified and measured in various brain areas of the rat (3 months old). Its concentrations are (nmoles/g w.w.): Cortex 2.1±0.2, Hippocampus 1.1±0.2, Diencephalon 0.95±0.2, Cerebellum 0.70±0.3. The administration of p-chlorophenylalanine (300 mg/Kg 3 days before) reduces brain QA content by 40%. In newborn animals the cortical content of QA are (nmoles/g w.w.): 0.66±0.15. In the cortex of 30-month-old animals (aged rats) the concentration of QA is extremely variable: 2 out of 5 animals had a cortical content of this toxin higher than 7 nmoles/g w.w. The average value ± SE of 5 aged rats was 4.5±1.5 nmoles/g w.w. In order to test the regulation of QA synthesis, its precursor tryptophan (400 mg/Kg i.p.), has been administered both to adult and to newborn rats. Two h later the concentration of QA was measured in the cortex, and those of 5-HT (another tryptophan metabolite) in the basal ganglia. Tryptophan administration increases by 150% the content of QA in the adult cortex, but it does not change that in the cortex of newborn animals. On the other hand the content of 5-HT increases by approximately the same degree both in adult and in newborn basal ganglia, thus ruling out problems of tryptophan transport in the newborn brain. The present data demonstrate that QA is present and unevenly distributed in the rat brain. They also suggest that QA synthesis from tryptophan is greater in adult than newborn animals. Finally, in aged rats the QA content seems to increase. Possible changes in the content of QA could help to explain the neuronal degeneration which occurs in several neurodegenerative disorders and possibly also in pathological aging.

344.2 DETERMINATION OF QUINOLINIC ACID IN RAT AND HUMAN BRAIN.
M. Wolfensberger*, U. Amsler*, M. Cuénod (SPON: E. Perret), Brain Res. Inst., Univ. Zurich, Switzerland; W.O. Whetsell Jr., Univ. Tennessee, Ctr. of Health Sci., Memphis, TN 38163; A.C. Foster and R. Schwarcz, Md. Psychiatric Research Center, Baltimore, MD 21228.

We have previously reported (Science 219:318, 1983) that neurotoxic effects reminiscent of those observed in human neurodegenerative disorders occur following intrastriatal or intrahippocampal injections in the rat of the hepatic tryptophan metabolite quinolinic acid (QUIN; 2,3-pyridine dicarboxylic acid). To assess the presence and distribution of QUIN in the CNS we have now developed a methodology suitable for its trace analysis in rat brain tissue and autopsy material from human subjects.

200 mg tissue was sonicated in 20 vols. (w/v) 6% perchloric acid and 2,4-pyridine dicarboxylate added as an internal standard. After removal of precipitated material, the neutralized supernatant was purified by anion (Dowex AG 1X8, Cl^--form) followed by cation (Dowex 50W, H^+-form) exchange chromatography. The final eluate was dried down, resuspended in water and further purified by HPLC using a Partisil PSX 10/25 SAX anion exchange column. The fractions containing QUIN and the internal standard were combined, dried and extracted with methanol. The methanol fraction was dried and the pyridine dicarboxylates converted to the corresponding di-hexafluoroisopropyl esters. These derivatives were dissolved in toluene and 2 µl-aliquots applied by splitless injection onto a glass capillary column (20 m x 0.3 mm, methylphenyl siloxane, 0.2 µm film thickness) connected to a mass spectrometer. The derivatives were chromatographically separated and detected by single ion monitoring at m/z 272.

QUIN was unequivocally identified in both rat and human brain tissue. Rat cerebellum, striatum and frontal cortex contained 434 ± 64, 759 ± 289 and 1584 ± 265 fmol/mg wet tissue weight, respectively (N=6). In normal human subjects (N=6), values were 576 ± 165, 369 ± 55 and 567 ± 126 fmol/mg tissue for cerebellum, caudate nucleus and frontal cortex, respectively.

Supported by SNSF grants 3.228.82 and 3.506.79 and by USPHS grants NS-16102 and 16941.

344.3 EVIDENCE IN SUPPORT OF A NEURONAL LOCALIZATION OF N-ACETYL ASPARTYL GLUTAMATE: REGIONAL BRAIN DISTRIBUTION AND THE EFFECTS OF LESIONS.
K.J. Koller, R. Zaczek*, and J.T. Coyle. Dept. of Neuroscience, Dept. of Psychiatry, Div. Child Psychiatry, Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

The endogenous acidic amino acids L-glutamate (GLU) and L-aspartate have excitatory effects when applied to neurons in the mammalian CNS and may act as excitatory amino acid neurotransmitters. Recently we have described an endogenous peptide, N-acetyl-aspartyl-glutamate (NAAG), which is composed of these two amino acids and acts at a subpopulation of [^{3}H]L-GLU binding sites (Zaczek, et al, PNAS, 1983,80:1116). In the present study, we have developed an HPLC method to measure endogenous NAAG and N-acetyl-aspartate (NAA) levels in tissue.

Immediately upon dissection, tissue from male Sprague-Dawley rats was sonicated in 10 vol ice-cold methanol:H_2O(9:1). After centrifugation, primary amines were removed by chromatography over AG-50. The void volume was lyophilized and assayed via an isocratic HPLC system (Whatman Partisil-10 SAX column; absorbance at 214 nm; 0.1 M KH_2PO_4 + 0.025 M KCl mobile phase). N-acetyl-aspartate had a retention time of 6.2 min and NAAG of 15 min. Sixteen regions of the CNS as well as the heart and liver were assayed for NAA and NAAG. Except for pituitary (0.37 ± 0.8 nmol/mg prot), striatum exhibited the lowest concentration of NAAG (2.01 ± 0.1 nmol/mg prot) while the levels in the spinal cord were considerably higher than any other region (25.2 ± 1.4 nmol/mg prot). NAAG was undetectable in heart and liver. NAA concentrations averaged 6-fold greater than those of NAAG with the highest concentration in the frontal cortex (67.6 ± 6.2 nmol/mg prot). There was a poor correlation (r=0.17;N=16) between the regional distributions of NAA and NAAG.

One week after decortication, a 28±4% reduction in NAAG levels in the ipsilateral striatum was observed while no significant reduction occurred contralaterally. Injection of 3.25 nmol kainic acid (KA) into the striatum caused a 30 ± 5% decrease in striatal NAAG. KA lesion (3.25 nmoles) of the dorsal hippocampus resulted in a 37±4% decrease in NAAG in the ipsilateral hippocampus with no significant decrease contralaterally; a 31±4% reduction of NAAG was also observed in the septum. Transection of the spinal cord at mid-thorax reduced NAAG levels both rostral and caudal to the lesion (51±3%, 57±3%, respectively).

The results are consistent with a neuronal localization of NAAG within the CNS and possibly within cortical efferent systems such as the cortico-spinal tract. Since NAAG is an excitatory peptide with high affinity for a subset of glutamate receptors, it may serve as an excitatory neurotransmitter.

344.4 QUINOLINIC ACID ACTS AT NMDA-TYPE RECEPTORS ELICITING [^{3}H]-ACETYLCHOLINE RELEASE FROM STRIATAL SLICES.
J. Lehmann, P. Schaeffer*, J.W. Ferkany and J.T. Coyle. Depts. of Neuroscience, Psychiatry, and Pharmacology, Div. Child Psychiatry, Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

Excitatory amino acids evoke the release of [^{3}H]-acetylcholine formed from [^{3}H]-choline in striatal slices by interacting with the type of excitatory amino acid receptor which is selectively activated by N-methyl-D-aspartate (NMDA; Lehmann and Scatton, Brain Res. 252:77, 1982). Quinolinic acid (QUIN) has been described as a potent agonist at these NMDA-type receptors (Stone and Perkins, Eur. J. Pharm. 72:411, 1981). Rat striatal slices (300um) were incubated for 30 min in magnesium-free Krebs medium gassed with 95:5% O_2:CO_2 containing 50nM [^{3}H]choline (78 Ci/mmol). The slices were placed in perfusion chambers and superfused with oxygenated Krebs medium at 0.7 ml/min. To inhibit the re-uptake of [^{3}H]choline, 10 uM hemicholinium-3 was included in the medium.

QUIN (0.5 mM to 7.5 mM) evoked the release of [^{3}H]-acetylcholine formed from [^{3}H]-choline from superfused rat striatal slices. The release of [^{3}H]-acetylcholine evoked by QUIN was greatly attenuated by the presence of magnesium (1.2 mM) in the superfusion medium, a characteristic also of [^{3}H]-acetylcholine release evoked by other NMDA-type receptor agonists. The maximal release of [^{3}H]-acetylcholine evoked by 2 min pulses of QUIN was 214% of baseline, compared to a maximal increase by NMDLA of 271%, suggesting similar efficacy of the two compounds. QUIN was found to have an EC_{50} of 2 mM, forty-fold weaker than that of NMDLA (EC_{50} = 50uM). The EC_{50} of ibotenic acid has been previously reported also to be 50 uM (Lehmann and Scatton, 1982).

The release of [^{3}H]-acetylcholine evoked by QUIN (2 mM) was antagonized by D-2-amino-5-phosphonopentanoic acid and by DL-2-amino-7-phosphonoheptanoic acid, with IC_{50} values of 10 uM and 25uM, respectively. These antagonists of NMDA-type receptors have identical IC_{50} values when tested against the EC_{50} of NMDLA. Glutamate diethylester (GDEE), an antagonist of quisqualate-preferring receptors, and DL-2-amino-4-phosphonobutyric acid, each tested at 250 uM, had no effect on [^{3}H]-acetylcholine release evoked by QUIN (2 mM).

These results suggest that QUIN acts at NMDA-type receptors in the striatum to evoke [^{3}H]-acetylcholine release. Despite the 40-fold lower potency of QUIN than the agonists NMDLA and ibotenate, the action of QUIN appears quite specific at these concentrations in view of its sensitivity to magnesium and antagonists of NMDLA-type receptors.

344.5 KYNURENIC ACID, AN ENDOGENOUS METABOLITE, SELECTIVELY ANTAGONISES QUINOLINATE NEUROTOXICITY. A.C. Foster and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228.

Kainic (KA) and ibotenic (IBO) acids are two heterocyclic excitotoxins of plant or fungal origin which have been used to provide animal models of certain neurodegenerative disorders (Neurosci. Res. Prog. Bull. 19, 331, 1981). Quinolinic acid (QUIN), a tryptophan metabolite in mammalian liver whose presence has also been demonstrated in rat and human brain tissue (Wolfensberger et al., this meeting), causes similar excitotoxic lesions after intracerebral application. Since blockade of QUIN-induced nerve cell loss is of considerable theoretical and possibly therapeutical interest, we have begun to examine potential QUIN-antagonists. Kynurenic acid (KYNA) seemed an interesting candidate since it blocks neuronal excitation caused by QUIN (Brain Res. 247, 184, 1983) and is also a metabolite of tryptophan in mammalian liver.

Light microscopic analyses revealed that co-injection of KYNA and QUIN (ratio 3:1) into the rat striatum or hippocampus completely prevented the development of neuronal lesions. This effect was specific for KYNA since no protection was observed after co-administration of QUIN and either xanthurenic or quinaldic acids, two close structural analogs of KYNA. Quantitative assessment of KYNA's antagonistic potency was made by measuring choline acetyltransferase (CAT) activity following striatal co-injections of 240 nmol QUIN with varying amounts of the antagonist: a steep dose-effect curve ranged from no protection at 106 nmol KYNA to 78% protection at 212 nmol KYNA.

The activity of KYNA was also tested against KA- and IBO-induced neurotoxicity: doses of KA (10 nmol) or IBO (150 nmol), leading to an approx. 50% loss of striatal CAT, were co-injected with KYNA (1058 nmol). KYNA partially (by 43%) attenuated the toxic effect of KA but failed to block that of IBO. Thus, KYNA's pharmacological profile contrasts that observed for the synthetic antagonist, (-)2-amino-7-phosphonoheptanoic acid, a potent blocker of QUIN- and IBO-induced (but not KA-induced) neurotoxicity.

The presence of KYNA, a tryptophan metabolite in the periphery, has not been reported in CNS tissue. In the liver, kynurenine transaminase (KYNTase) synthesises KYNA from kynurenine. Following an earlier report (J. Neurochem. 23, 271, 1974), we have found KYNTase activity to be rather similar in 6 regions of the rat forebrain (0.2 nmol/mg tissue/hr at a substrate concentration of 2 mM) and 2-fold greater in the cerebellum. Since its enzymatic precursor kynurenine is an established CNS constituent, the biosynthesis of KYNA in brain tissue thus appears plausible. KYNA's ability to influence endogenous excitotoxic phenomena and its likely presence in the CNS justify a thorough examination of its role in normal and abnormal brain function.

Supported by USPHS grants NS 16102 and 16941.

344.6 EFFECTS OF EXCITATORY AMINO ACIDS ON DOPAMINE SYNTHESIS IN THE RAT RETINA *IN VIVO*. W.W. Morgan and C.W. Kamp. Dept. of Anatomy, The University of Texas Health Science Center, San Antonio, TX 78284.

Intraocular administration of 10 nmole quisqualate but not of N-methyl-D-aspartate or kainate significantly augmented dopamine synthesis in the retinas of dark-maintained rats. The stimulation evoked by quisqualate was almost as great as that shown previously to be evoked by light exposure. Dihydroxyphenylalanine accumulation subsequent to inhibition of L-aromatic amino acid decarboxylase by m-hydroxybenzylhydrazine was used to estimate dopamine synthesis *in vivo*. The effect of quisqualate was dose related, but the putative glutamate antagonist, glutamate diethyl ester (GDEE, 60 nmole/eyeball), was unable to block the action of quisqualate. Furthermore, dosages of GDEE up to 240 nmole/eyeball produced no suppression of the light-induced enhancement of retinal dopamine synthesis. These results are the first to show that the quiescent dopamine neurons of the dark-adapted rat retina can be activated by an excitatory amino acid analogue of the putative endogenous neurotransmitter, glutamate. The fact that GDEE was unable to reduce either quisqualate- or light-enhanced dopamine synthesis might be interpreted as evidence that quisqualate is not acting via a glutamate receptor. Further, the inability of GDEE alone to suppress the light-induced increase in dopamine synthesis suggests that glutamate may not have a physiological role in activating the dopamine neurons in the light. On the other hand the selectivity of GDEE as a glutamate receptor antagonist is not universally accepted (McLennan, Adv. Biochem. Psychopharmacol. 27, 253, 1981). Project supported by a Pharmaceutical Manufacturers Association Foundation Starter Grant to CWK and DA 00755 and Research Scientist Development Award DA 00083 to WWM.

344.7 STUDIES ON THE CEREBRAL UPTAKE OF QUINOLINIC ACID AFTER INTRACEREBRAL OR SYSTEMIC ADMINISTRATION. L.P. Miller*, L. Braun*, W. Oldendorf, A.C. Foster and R. Schwarcz (SPON: A. Morin). Brentwood VA Hospital, Los Angeles, CA 90073 and Maryland Psychiatric Research Center, Baltimore, MD 21228.

Following our previous observations on the selective neurotoxic effects of intracerebral quinolinic acid (QUIN; Science 214: 318, 1983), we have investigated the persistence of QUIN after its intrastriatal application. For this purpose, 1μCi ^{3}H-QUIN (Nuclear Research Center, Negev, Israel; 0.5 nmol) was injected unilaterally into the striatum and rats killed at various time intervals. After 5 min, 29% of the applied radioactivity remained within the injected striatum, decreasing to 20% after 30 min and to 6% at 120 min (N=3 each). Using ion exchange and HPLC methodologies, the identity of the recovered radioactivity at each of the three time points corresponded exclusively to unmetabolized QUIN. The rapid disappearance and apparent lack of in vivo degradation of QUIN after intracerebral infusion suggested an inability of striatal cells to accumulate QUIN from extracellular space. In vitro experiments with 0.5 mm thick striatal slices exposed to 10^{-4}M or 10^{-7}M ^{3}H-QUIN (100 nCi, 10 min, 25°C) supported this conclusion since no net uptake was observed after subtraction of blank values (zero Na^+ or 4°C).

In view of the neurotoxic and convulsive properties of intracerebral QUIN (Brush et al., this meeting) and the established presence of QUIN in peripheral organs, the possibility must be considered that abnormally high blood levels of QUIN may have neuropathological consequences. In this regard, systemic administration of large doses of QUIN has been shown to cause convulsions in rodents and elevated peripheral QUIN-levels have been speculatively linked to the etiology of human epilepsies (Epilepsia 22, 257, 1981). We have monitored behavioral and EEG patterns before, during and after i.v. infusion of 450 mg/kg QUIN in unanesthetized rats. Compared to control animals (infused with equimolor amounts of nicotinic acid), no convulsions or other behavioral abnormalities were noticed following QUIN administration. However, after a latency period of 15-20 min, 4 out of 5 rats displayed brief spiking episodes, which were mostly restricted to the hippocampal formation. In no case did we observe the characteristic repetitive seizure pattern or neuropathology triggered by intrahippocampal QUIN. Thus, it seems that only marginal amounts of QUIN enter the brain under our experimental conditions. To make a more quantitative assessment, QUIN uptake into the brain was studied by the Oldendorf-technique (Res. Meth. Neurochem., 5, 1981). Using a dose equivalent to that employed in the EEG-experiment, <0.4% of QUIN was found to cross the blood-brain barrier.

Supported by USPHS grant NS 16102.

344.8 EFFECT OF L-CANALINE ON ORNITHINE AMINOTRANSFERASE ACTIVITY AND GLUTAMATE CONTENT IN THE SEPTUM. J.T.Wroblewski W.D. Blaker J.L. Meek Lab. Preclinical Pharmacology, NIMH, St. Elizabeths Hosp. Washington D.C. 20032

Ornithine aminotransferase (OAT) may be involved in synthesis of neurotransmitter gluatamate as suggested by lesion and regional distribution studies (Wong, McGeer, McGeer, 1982). In vitro, radioactive ornithine can be converted to glutamate by synaptosomal preparations. We investigated how changes in OAT activity affect glutamate content in vivo. L-Canaline, which is a potent, specific, irreversible inhibitor of OAT in vitro was injected intraseptally into rats. The septum was chosen due to its well defined glutamatergic input from the hippocampus. OAT was measured by the rate of glutamate formation from ketoglutarate and ornithine by HPLC. Canaline produced a dose dependent decrease of OAT activity and glutamate content in the septum. At a 100 ug dose, OAT inhibition reached 90% in 5 min and persisted for at least 1 h. Glutamate decreased rapidly for 20 min with a half life of 7 min, and more slowly for the next 2 hrs (to 30% of control) with a half life of 8 h. The nature of the fast pool, amounting to about 20% of total glutamate was investigated by performing a fimbria-fornix transection. Rats were injected with canaline 2 h after the lesion and glutamate was measured at intervals up to 20 min. The lesion, which by itself resulted in an 8% decrease of septal glutamate, produced a 3-fold increase in the apparent half life of the rapidly changing pool of glutamate. This indicates that OAT is involved in the maintenance of a glutamate pool which is sensitive to an acute lesion of the glutamatergic input. Canaline also reduced septal glutamate content 8 d after fimbria-fornix lesion, when the degeneration of glutamatergic nerve terminals may be expected, indicating that OAT is not limited to the production of nerve terminal glutamate. Our experiments demonstrate, however, its the participation of OAT in the synthesis of this pool. These data are consistent with earlier suggestions that ornithine may serve as a precursor of the neurotransmitter glutamate.

344.9 ACIDIC AMINO ACID RECEPTORS IN POSTSYNAPTIC DENSITIES.
G.E. Fagg* and A.I. Matus. Friedrich Miescher Institute, P.O. Box 2543, CH-4002 Basel, Switzerland.

Na^+-independent synaptic membrane binding sites for the excitatory neurotransmitter L-glutamate recently have been subdivided into two distinct classes which differ in their Cl^- and Ca^{2+} requirements and their pharmacological specificity (Fagg et al., J. Neurosci. 2, 958, 1982; Eur. J. Pharmac. 88, 105, 1983). Both sites display characteristics suggesting receptor roles at acidic amino acid-using synapses. In order to further evaluate the functions and regulation of these sites at the postsynaptic membrane, we have examined the properties of L-^{3}H-glutamate binding to isolated postsynaptic densities (PSDs).

PSDs were prepared from synaptic plasma membranes (SPMs) by treatment with 1% Triton X-100 and centrifugation through 1.0 M sucrose, and L-glutamate binding was assayed at 30°C in 50 mM Tris acetate buffer. Binding was optimal at physiological pH, and exhibited saturation kinetics, with a Kd of approximately 0.5 µM. In common with well-established receptor systems, but in contrast to Cl^-/Ca^{2+}-dependent L-glutamate binding to SPMs, binding to PSDs also occurred at 4°C, although a longer time was required to reach equilibrium. Cl^- was a less potent stimulant of L-glutamate binding to PSDs than to SPMs, although a number of divalent cations (1 mM) were active in this respect. Concentrations of Na^+ (1-5mM) which inhibit Cl^-/Ca^{2+}-dependent L-glutamate binding to SPMs were without effect on the binding of L-glutamate to PSDs, both in the presence and absence of Cl^- and Ca^{2+}.

Structure-activity studies indicated that the L-isomers of glutamate and aspartate were potent inhibitors of L-^{3}H-glutamate binding to PSDs, with glutamate slightly more effective than aspartate. The D-isomers of both amino acids were less potent, although stereoselectivity was less marked in the case of aspartate. Ibotenate, N-methylaspartate and quisqualate were moderately effective displacers, and kainate was weaker. Of the α-amino-ω-phosphono-carboxylic acid homologues, the valerate derivative was the most powerful displacer, as observed for Cl^-/Ca^{2+}-independent sites in SPMs. The butyrate analogue, which is a potent inhibitor of Cl^-/Ca^{2+}-dependent binding in SPMs, was weaker in PSDs.

These data demonstrate that, as in the case of GABA receptors (Matus et al., J. Neurobiol. 12, 67, 1981), only a subpopulation of SPM-located L-glutamate binding sites is found at the postsynaptic density. Kinetic, ionic and pharmacological considerations indicate that these sites are not of the Cl^-/Ca^{2+}-dependent class which predominates in SPMs, but that they have features in common with Cl^-/Ca^{2+}-independent SPM binding sites. The isolation and molecular characterization of these sites is currently in progress.

344.10 AUTORADIOGRAPHIC LOCALIZATION AND CHARACTERIZATION OF GLUTAMATE RECEPTORS IN RAT BRAIN. S. Halpain, B. Parsons and T.C. Rainbow. The Rockefeller University, New York, NY 10021 and Dept. of Pharmacology, Univ. of Penn. Sch. of Med., Philadelphia, PA 19174.

Several laboratories recently have described sodium-independent binding sites for glutamic acid (Glu), a putative neurotransmitter at several excitatory pathways. These binding sites have many properties expected of post-synaptic receptors for glutamate or glutamate-like compounds. We have applied the technique of quantitative autoradiography in an effort to localize these receptors in brain and describe their regional properties.

Thaw-mounted cryostat sections (32 µ) were prepared and processed for autoradiography on tritium-sensitive film according to methods described previously (Eur.J.Pharm. 86:313; J.Neurosci. Meth. 5:127). Scatchard analysis yielded an apparent K_d of 714 ± 189 nM and B_{max} of 0.46 ± 0.024 pmol/mg protein (ave. for whole brain section). Kinetic studies gave comparable results, with an association rate constant of about $0.12 \mu M^{-1} min^{-1}$ and dissociation rate constant of about 0.20 min^{-1}. L-Glu was the strongest inhibitor of the binding, but several acidic amino acids also showed high affinity for the site (in order of increasing potency): L-quisqualate, L-aspartate, DL-homocysteate, L-cysteine sulfinate. Ibotenate was moderately effective in inhibiting binding. D-Glu, kainic acid, aminophosphono butyric acid and the L- and D-isomers of α-amino adipic acid caused little or no displacement. The putative glutamate antagonist glutamate diethylester (GDEE) was a strong displacer of ^{3}H-Glu binding. Binding was not markedly affected by 0.5mM Ca++ but was enhanced by 20mM Cl^-.

Specific binding appeared greatest in telencephalic areas (amygdala, striatum, hippocampus, neocortex) and in cerebellar cortex. Diencephalon showed high to moderate binding, with clear differences among thalamic and hypothalamic nuclei. Midbrain exhibited low levels of specific binding. Caudal, brainstem (pons, medulla, and associated nuclei) and white matter areas were not distinguishable from background levels. Laminations were visible in cerebellum (granule > Purkinje > molecular), neocortex (outer layers > inner layers > layer IV), and hippocampal formation (dentate gyrus > stratum radiatum > stratum oriens > hilar region). The highest levels of binding were found along the hippocampal perforant path and Schaffer collateral/commissural pathways, thus supporting the idea that these are major glutamatergic projections. Recently several groups have proposed the existence of multiple glutamate receptors on the basis of divergent pharmacological profiles under certain conditions. The binding sites we label with ^{3}H-Glu on frozen tissue sections share some of the properties described by others using synaptic membrane preparations, but our results differ in some aspects from all the Glu receptors so far defined. (Supported by NS19597,NS20006 & a Sloan Fellowship to TCR)

344.11 MULTIPLE BINDING SITES FOR L-[^{3}H]GLUTAMATE ON HIPPOCAMPAL SYNAPTIC MEMBRANES: EFFECTS OF CALCIUM AND CHLORIDE IONS. Linda L. Werling and J. Victor Nadler. Dept. Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Our attempts to identify and characterize glutamate receptors in binding studies on rat hippocampal synaptic membranes have yielded evidence of three binding sites with K_D values for L-glutamate of 12, 200 (GLU A) and 1000 (GLU B) nM. GLU A binding is the fraction of bound radioligand that is displaced by 5 µM quisqualate, whereas GLU B binding persists in the presence of 5 µM quisqualate and is displaced by 100 µM ibotenate. Effects of selective lesions suggest that in fascia dentata GLU A binding sites are localized mainly on the dentate granule cells, whereas GLU B binding sites are localized, in part, on the perforant path fibers, but not on granule cells.

Other workers have reported the enhancement of glutamate binding by Ca^{2+} and Cl^-, but have not analyzed the effects of these ions on individual binding sites. Binding of 20 nM L-[^{3}H]glutamate to the GLU A site was about 20 times as great in 50 mM tris-HCl buffer as in tris-HOAc buffer. Addition of Ca^{2+} to tris-HCl buffer slightly inhibited GLU A binding at concentrations of 3.9-13 µM and stimulated at concentrations above 39 µM. Maximal (2.4-fold) stimulation was obtained with a concentration of 0.39 mM. The percentage stimulation by 1.3 mM Ca^{2+} (2.2-fold) was the same in tris-HCl or tris-HOAc buffer. The stimulatory effect of 0.4 mM Ca^{2+} was accounted for by an increase in the maximum density of binding sites with no change in their affinity for L-glutamate. Binding of 20 nM L-[^{3}H]glutamate to the GLU B site was only 2-3 times as great in 50 mM tris-HCl buffer as in tris-HOAc buffer. In tris-HCl buffer Ca^{2+} maximally stimulated GLU B binding (30-40%) at concentrations of 0.13-3.9 mM, but Ca^{2+} had little effect in tris-HOAc buffer. Cl^- (47 mM) increased the maximum density of GLU B binding sites without altering their affinity for L-glutamate. In the presence of 0.4 mM Ca^{2+} GLU B Scatchard plots became curvilinear, moreso in tris-HCl than in tris-HOAc buffer. Resolution of these curves suggested that Ca^{2+} increased the maximum density of GLU B binding sites only in tris-HCl buffer and little affected their affinity for L-glutamate. In addition, inclusion of Ca^{2+} in either buffer permitted detection of a novel binding site (or state) with a K_D for L-glutamate of about 5 µM.

These results suggest that GLU A binding is essentially Cl^--dependent and stimulated by Ca^{2+}, whereas GLU B binding is stimulated to a lesser degree by both ions, but requires neither. In addition, hippocampal synaptic membranes appear to contain an essentially Ca^{2+}-dependent, Cl^--stimulated binding site with micromolar affinity for L-glutamate. (Supported by NIH grant NS 16064).

344.12 PIPECOLIC ACID BINDING TO RAT BRAIN MEMBRANES. Maria d.C. Gutierrez* and Ezio Giacobini, Dept. Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62708

The imino acid, pipecolic acid (piperidine-2-carboxylic acid, PA) represents the major product of lysine metabolism in the mammalian brain (Giacobini, E. et al., Cell. Mol. Biol., 26:135, 1980). Evidence for a specific neuromodulatory role of PA obtained in our laboratory includes: its occurrence and biosynthesis, its distribution and selective accumulation in brain; its high affinity, its Na^+-dependent uptake in synaptosomes, its Ca^{++}-dependent release from brain slices following K^+ stimulation and the presence of a high affinity binding to mouse brain (K_D = 45 nM). We have now determined and characterized ^{3}H-PA binding to rat brain. Fresh crude P_2 fraction membranes were incubated for 60 (Na^+-indep.) or 120 (Na^+-depend.) min at 0°C in the presence of various concentrations of ^{3}H-PA in the nanomolar range. Non-specific binding was determined by incubation in the presence of 1 mM of unlabelled PA. The binding was found to be saturable, heat sensitive and Na^+-independent. A high affinity binding site with an apparent K_D = 32 nM was demonstrated. No binding was present in membranes derived from cerebellar fractions. The displacement of ^{3}H-PA binding with PA and GABA analogues was studied. Our results suggest that in the rat brain, as in the mouse, there is a specific binding site for ^{3}H-PA. This result supports the hypothesis of a neuromodulatory role of PA in mammalian brain.

344.13 AGONIST AND ANTAGONIST PROPERTIES OF KYNURENIC ACID AND QUINOLINIC ACID IN RAT HIPPOCAMPAL SLICES. A. H. Ganong*, T. H. Lanthorn, and C. W. Cotman, Dept. of Psychobiology, Univ. of California, Irvine, CA 92717.

Kynurenic acid, a tryptophan metabolite, has been shown to inhibit responses in cortical neurons induced by ionophoretic application of excitatory amino acids, including the tryptophan metabolite quinolinic acid. The present results show that kynurenic acid blocks synaptic transmission in excitatory pathways in hippocampus, possibly by blocking postsynaptic receptors of putative amino acid mediated synapses. We have also shown that quinolinic acid depolarizations can be blocked by kynurenic acid and other N-methyl-D-aspartate (NMDA) antagonists and that quinolinic acid produces excitatory responses similar to those which have been reported for NMDA.

Extracellular field potentials produced by stimulation of the lateral perforant path, the mossy fibers, or the Schaffer collateral-commissural pathway were reduced by approximately 50% in the presence of 500-1000 μM kynurenic acid. Intracellular recordings from CA1 pyramidal cells showed that inhibition of Schaffer collateral commissural EPSPs was not accompanied by changes in input resistance or resting membrane potential.

Perfusion of kynurenic acid also antagonized extracellular focal depolarizations induced by ionophoretic application of excitatory amino acids in stratum radiatum of hippocampal slices. Half-inhibition of focal depolarizations induced by NMDA, ibotenic acid, and quinolinic acid occurred with perfusion of 50-200 μM kynurenic acid. Kynurenic acid was a less effective blocker of focal potentials produced by kainate, L-glutamate, and quisqualate. Quinolinic acid focal potentials were also inhibited by the NMDA antagonists D-2-amino-5-phosphonovalerate and D,L-2-amino-7-phosphonoheptanoate. Perfusion of 5-10 μM solutions of these antagonists resulted in about half-inhibition of quinolinic acid responses.

Excitatory actions produced by ionophoretic application of quinolinic acid were further investigated by obtaining intracellular recordings from CA1 pyramidal cells. Application of quinolinic acid resulted in depolarization and apparent increases in input resistant as measured by injection of hyperpolarizing current pulses. Quinolinic acid depolarizations which reached firing threshold frequently resulted in bursts of action potentials compared to steady firing produced by L-glutamate. In the presence of TTX, quinolinic acid still induced depolarizations, apparent input resistance increases, and could elicit regenerative spikes.

MEMBRANE BIOPHYSICS II

345.1 CALCIUM DEPENDENCE OF RESTING NEURONAL CONDUCTANCE. J. W. Johnson* and S. H. Thompson* (SPON: U. J. McMahan). Hopkins Marine Station, Stanford Univ., Pacific Grove, CA 93950.

The membrane conductances of a resting neuron determine its rest potential, and in part what input can initiate an action potential. They therefore play a central role in neuronal integration. The ionic conductances which make significant contributions to resting cell conductance, however, are not well characterized. This is so partly because very small ionic currents are important to the low-conductance resting membrane, and direct measurement of such small currents is difficult due to inherent microelectrode noise. A simple technique was developed for quickly (~100 msec) generating low noise current versus voltage (I(V)) curves in a restricted voltage range (-40 to -80 mV) for a neuron under voltage clamp. This technique was used to study the contribution to resting cell conductance of membrane conductances dependent on internal Ca. Neurons from the buccal and abdominal ganglia of Aplysia california were used.

Internal Ca concentration was lowered by one of two methods: 1) Injection of the Ca chelator EGTA into the neuron (Ahmed & Connor, J. Gen. Physiol., 75: 403, 1980), or 2) Partial block of any resting Ca flux with $2mM$ Mn (Gorman & Thomas, J. Physiol., 308: 259, 1980) (direct effects on resting Ca conductance must be considered in this case). The effect of such treatment on membrane conductance was measured by subtraction of an I(V) curve measured before treatment from one measured afterwards. The difference I(V) curve reflects only any conductance changes induced by the treatment. If an affected Ca dependent conductance has little or slow voltage dependence in the range studied, the subtracted I(V) curve is effectively instantaneous for that conductance. The slope of the I(V) curve will reflect the magnitude of the change (most importantly whether there was a conductance increase or decrease). The zero current crossing is the apparent reversal potential of the affected conductance.

The effect of lowering internal Ca concentration varies considerably from cell to cell. In many Aplysia neurons, the dominant effect is a decrease in conductance with reversal potential near -60 mV, suggesting that in those cells significant Ca dependent K conductance is on at rest. In other neurons a decrease in internal Ca concentration results in a conductance increase with a reversal potential above 0 mV. Ca dependent conductances activated at rest may provide an important source of variability in neuronal excitability.

345.2 MECHANISM OF POSTINHIBITORY REBOUND IN MOLLUSCAN NEURONS. B. F. Jones*, J. W. Johnson* and S. H. Thompson* (SPON: J. K. Ono). Hopkins Marine Station, Stanford University, Pacific Grove, Ca. 93950.

Postinhibitory rebound (PIR) is a neuronal process which has been observed in a variety of experimental preparations (Perkel and Mulloney, Science, 185:181,1974). Neurons which have PIR exhibit a slowly decaying increase in spike frequency following cessation of an imposed hyperpolarization. We studied PIR in isolated neuronal somata from the buccal and abdominal ganglia of Aplysia californica. Using computer subtraction of voltage clamp current records to eliminate contamination by the transient potassium current (I_A), we have identified the ionic current responsible for the state of increased excitability characterizing PIR.

The current underlying PIR is time and voltage dependent. It is activated by hyperpolarizing voltage steps from holding potentials more positive than -50 mV. The current is not activated by pulses less than 2 sec in duration. The inward tail current following a 10 sec step from -40 to -50 mV has a peak value between 0.1 and 1.0 nA, and has a total duration of 20 to 30 sec. There is marked variability in the magnitude and time course of the response in different neurons studied. The current is sensitive to changes in the external concentration of potassium (K^+) ions, and it is blocked by addition of 2 mM nickel to the bathing medium (a treatment which interferes with tonic calcium (Ca^{++}) current).

The conductance change associated with PIR was studied using an instantaneous current-voltage (I(V)) curve subtraction technique (see adjacent abstract by Johnson and Thompson). We found that the inward PIR current is caused by a conduction decrease and that the apparent reversal potential of the process corresponds with the measured reversal potential for the Ca^{++} activated K^+ current (I_C) in these cells (-60 to -70 mV).

Our findings indicate that the PIR current is caused by a hyperpolarization-induced decrease in the tonic level of I_C. This decrease in I_C is presumably due to slow changes in the Ca^{++} concentration near the inner membrane surface. A computer model (Smith and Thompson, submitted J. Physiol.) which included the major ionic conductances found in molluscan neurons, as well as a simple Ca^{++} regulation system (consisting of membrane pumping, and cytoplasmic buffering and diffusion), was employed to produce a PIR response quantitatively similar to that seen in real cells.

345.3 INACTIVATION OF CA^{++}-DEPENDENT K^+ CURRENT CAN OCCUR WITHOUT SIGNIFICANT CA^{++} CURRENT INACTIVATION. D.L. Alkon, J. Farley*, B. Hay*, and J. Shoukimas. Section on Neural Systems, Lab. of Biophysics, NINCDS-NIH, Marine Biological Lab., Woods Hole, MA 02543.

A depolarization-induced rise of intracellular Ca^{++} in Type B photoreceptors (Connor and Alkon, in preparation) suggested the presence of a voltage-dependent Ca^{++} current. In the presence of K^+ current blockers (10 mM 4-AP which blocks I_A, and 100 mM TEA which blocks the delayed rectifier), depolarizing commands more positive than 20 mV from a -60 mV holding potential in fact first elicited a transient voltage-dependent, inward current ($I_{Ca^{++}}$) and a slower, sustained outward current, ($I_{Ca^{++}\text{-}K^+}$). All records were leak-corrected using positive (small) and negative pulses. $I_{Ca^{++}}$ and $I_{Ca^{++}\text{-}K^+}$ were abolished by replacing external Ca^{++} with Cd^{++} (10 mM) in which the I-V relation approximated that of the leak current. $I_{Ca^{++}\text{-}K^+}$ decreased while $I_{Ca^{++}}$ increased and became more sustained after intracellular injection of EGTA or replacement of external Ca^{++} with Ba^{++}. $I_{Ba^{++}}$ increased with increasing external Ba^{++}, $I_{Ca^{++}\text{-}K^+}$ increased with increasing external Ca^{++}. In 300 mM external K^+, commands to 0 mV elicited $\sim$ maximal $I_{Ca^{++}}$ (2-5 nA), but no $I_{Ca^{++}\text{-}K^+}$ (Fig. 1c, d), since no K^+ currents (including I_A without 4-AP and TEA) flowed at the new value for E_{K^+}. $I_{Ca^{++}\text{-}K^+}$, unlike $I_{Ca^{++}}$, showed clear inactivation, with 2-3 min for complete recovery (Fig. 1). In 100 mM Ca_o^{++}, $I_{Ca^{++}}$ did inactivate, while $I_{Ca^{++}\text{-}K^+}$ inactivation was enhanced. Inactivation of $I_{Ca^{++}}$ and $I_{Ca^{++}\text{-}K^+}$ may be due to a direct effect of elevated Ca_i^{++} as was shown for I_A and I_{Na^+} (Alkon et al., Biophys. J. 40:245, 1982).

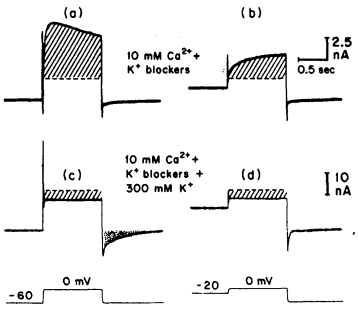


Fig. 1. Inactivation of $I_{Ca^{++}\text{-}K^+}$ without $I_{Ca^{++}}$ inactivation. (a) Step to 0 mV elicits outward current ($I_{Ca^{++}\text{-}K^+}$) above leak level (indicated by dashed lines). $I_{Ca^{++}\text{-}K^+}$ decreases during step. (b) 10-sec conditioning depolarization to -20 mV precedes a step to 0 mV, causing marked decrease of $I_{Ca^{++}\text{-}K^+}$. (c) As in (a) but with 300 mM K^+. Now inward current (below leak level), whose rising phase is not resolved here, does not decrease during step nor when preceded by conditioning depolarization in (d). (d) The inward tail, largely $I_{Ca^{++}\text{-}K^+}$, in (c) is eliminated in (d).

345.4 STIMULUS-INDUCED INTRACELLULAR Ca^{2+} TRANSIENTS IN APLYSIA NEURONS AND SQUID PRESYNAPTIC TERMINALS MEASURED WITH Ca^{2+}-SENSITIVE MICROELECTRODES. J.W. Deitmer[1]*, G.J. Augustine[1,3], R. Zucker[2] and R. Eckert[1]. Department of Biology, UCLA[1], Los Angeles, CA 90024, Department of Physiology-Anatomy, Univ. of Calif.,[2] Berkeley, CA, and Catalina Marine Science Center[3], Avalon, CA.

Single-barreled Ca^{2+}-sensitive microelectrodes (tip <3μm) containing ETH-1001 neutral carrier cocktail (Oehme et al., 1976, Chimica 30:204) were used to record changes in the intracellular Ca activity, aCa_i, in giant neurons (R2, L2-4) of Aplysia californica and in the presynaptic terminal of the giant synapse in the stellate ganglion of Loligo opalescens. Two additional electrodes were used to record membrane potential and inject current. In voltage-clamped Aplysia neurons, the Ca electrode had to be very close (<20μm) to the inner membrane surface to detect significant changes in aCa_i in response to depolarization, as reported by Levy et al. (1982, Biophys. J. 37:182a). This suggests that Ca changes are restricted to the vicinity of the cell membrane. With the electrode approximately 5 to 10 μm from the membrane, voltage clamp pulses (single 0.3-3s pulses or repetitive 0.1s pulses at 5 Hz) to +20 mV from -40 mV produced a rise in aCa_i of several tenths μM from a resting level of 0.1 to 0.5 μM. These increases in aCa_i were fully reversible, decaying with a half time of 3-8 s, while the electrode response time was less than 1 s. These signals presumably reflect Ca entry through voltage-gated Ca channels, because voltage steps to -100 mV or +100 mV did not produce any measurable signal, and transients were abolished in Ca-free saline or by addition of the Ca channel blocker Co.

In squid presynaptic terminals, aCa_i transiently rose in response to tetanic stimulation (10-100 action potentials/s for 1 to 5 s), declining with a half time of 3-8 s. Increasing extracellular Ca from 11 mM to 52 mM elevated resting aCa_i, and increased stimulus-induced transients in aCa_i. On the other hand, when the Ca electrode was placed into the postsynaptic giant axon no increase in aCa_i was detected even after long-lasting stimulation (100Hz spike trains lasting up to 10 s). Presynaptic Ca transients were absent in Ca-free saline and in the presence of Mn. In voltage-clamped presynaptic terminals 1-3s depolarizing steps to -10 mV elicited transient increases in aCa_i. These transients were smaller following depolarizations to potentials near E_{Ca}, and were absent following hyperpolarizations.

Supported by NSF BNS80-12346, USPHS NS8364, USPHS NS 07101, USPHS NS 15114, and a Max Kade Fellowship to J.W.D.

345.5 MEMBRANE PATCHES AND CELL MEMBRANES: A COMPARISON. A. Fox, J.M. Fernandez, and S. Krasne, Dept. of Physiol., Sch. of Med., Univ. of California, Los Angeles, Calif. 90024. (SPON: M. Barish.)

Rat clonal pituitary tumor cells (GH_3) were internally perfused and voltage clamped using the whole cell mode of the patch pipette technique. Internal solutions were either 120 mM N-methyl-glucamine fluoride or CsF, 11mM EGTA, 1mM $CaCl_2$, 2mM $MgCl_2$, and 10mM HEPES. All solutions were adjusted to pH 7.2 and 290 mOsm/kg. When the external solution was 150 mM NaCl, 2mM $CaCl_2$, 1mM $MgCl_2$, and 10 mM HEPES, large (typically a 600 pA max.) Hodgkin-Huxley-type, TTX sensitive sodium currents were observed. The peak Na^+ current vs. potential (Na^+ I-V) was measured shortly after beginning whole-cell recording and 1/2 hour later. The later Na^+ I-V was shifted by about 25 mV in the hyperpolarizing direction with respect to the earlier one, the Na^+ currents being otherwise identical. Outside-out patches of membrane were formed after about 1/2 hour of whole-cell recording. Single Na^+ channels were observed, and when averaged, the resulting currents superimposed on the late whole-cell Na^+ currents, indicating that formation of the patch did not alter the single Na^+ channel properties.

When the external solution was 150mM Tris-HCl (pH 7.2), 2mM $CaCl_2$, 1mM $MgCl_2$ and 10mM HEPES plus 300nM TTX, no ionic currents were observed. Addition of 5uM of the hydrophobic anion tetraphenyl borate (TØB) produced large displacement currents (600 pA max.) with a maximum decay time constant of 7 ms. The amount of TØB charge moved as a function of potential (q-V) increased sigmoidally in the potential range between -250 and +150 mV. The TØB q-V measured shortly after the beginning of whole-cell recording had a centerpoint of about 0 mV. The q-V's measured 1/2 hour and 1 hour later had centerpoints at -22 mV indicating that a shift in the TØB q-V had taken place in the first 1/2 hour of whole-cell recording comparable to that observed for the Na^+ currents. TØB currents before and after the shift were otherwise identical. TØB currents were measured in outside-out patches and were also observed to shift within a 1/2 hour period. The later patch recordings showed the same voltage-dependence for both the charge moved and time-constants as the late whole-cell recordings, indicating that patch formation does not alter translocation through the bulk lipid phases of the membrane. Shifts in the voltage-dependence of ionic and TØB displacement currents of the type described here may be related to the slow extrusion of large anions from the cell interior. Once these shifts are allowed for, however, patches of membrane and whole-cell membranes appear identical in their ionic channel and displacement current properties. --Supported by AHA fellowships to J.M.F. and A.F. and by NIH (HL20254) and MDA grants to S.K.

345.6 CELL SURFACE MORPHOLOGY AND THE PATCH CLAMP TECHNIQUE. J.M. Fernandez, A. Fox and S. Krasne, Dept. of Physiol., Sch. of Med., Univ. of California, L.A., Calif. 90024. (SPON: P. O'Lague)

Rat clonal pituitary tumor cells (GH_3) were cultured in 85% Ham's F-10, 15% horse serum, 2.5% fetal calf serum and 2.5% streptomycin under 5% CO_2 at 37 C. Cells were plated on Falcon petri dishes and removed from the incubator 2-3 days after plating for observation with the SEM. The cells were fixed in 2% gluteraldehyde in a buffered solution at pH 7.2 and 470 mOsm/kg. The cells had a diameter of 10-15 μm. SEM at 3000X or 8000X revealed a very rough surface comprised of microvilli, ruffles, blebs (up to 2 μm in diameter) and large numbers of fiber-like projections attaching the cells to the substratum or to other cells. With cells cultured as described above, we had nearly 100% success in obtaining gigaseals with low resistance patch pipettes, indicating that the observed surface features do not interfere with the formation of patches. In one case, cells were allowed to age for 5 days without replacing the medium and the number of gigaseals obtained dramatically decreased. When observed by SEM, these cells showed a marked decrease in surface features, suggesting that the extra membrane stored in the form of blebs, microvilli, etc. may be necessary for patch formation.

The capacitance as a function of frequency was measured using the whole-cell mode of the patch clamp technique in the absence of permeant ions (Internal: 120mM CsF, 11mM EGTA, 1mM $CaCl_2$, 2 mM $MgCl_2$ and 10mM HEPES. External: 150mM Tris-HCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 10mM HEPES, pH 7.2) using the pseudo-random binary signal technique. Typical cell DC capacitance was 8-12 pF after subtracting a pipette capacitance of 5-7 pF measured in the cell attached mode prior to whole-cell recording (both measurements performed in the same cell). When the apparent diameter of the cell was considered, the DC specific capacitance was about 2-3 $\mu F/cm^2$. Both the capacitance and the conductance were frequency dependent even after correcting for the series resistance in the pipette, ruling out a simple RC equivalent circuit for GH_3 cells.

SEM and capacitance measurements indicate that GH_3 cells have large amounts of extra surface membrane beyond that expected for simple spheres. Therefore, current densities should be standarized by capacitance and not by apparent area. Assumptions about space clamp in these or other mammalian cells are not straigthforward given their complex geometries; however, our successful reconstruction of the whole-cell inward sodium currents from single channel recordings in membrane patches (see abstract by A. Fox, J.M. Fernandez and S. Krasne), suggests that for low current densities there are no significant deviations from the space clamp assumption in GH_3 cells. --Supported by AHA fellowships to J.M.F. and A.F. and by NIH and MDA grants to S.K.

345.7 RECORDINGS OF SINGLE GABA-ACTIVATED CHANNELS FROM ACUTELY-ISOLATED HIPPOCAMPAL NEURONS. Richard Gray, Judianne Kellaway* and Daniel Johnston. Neuroscience Program and Neurology Department, Baylor College of Medicine, Houston, Texas 77030.

Considerable interest has been focused on the membrane and synaptic properties of hippocampal neurons from the in vitro slice preparation. For a number of technical and theoretical reasons, however, detailed biophysical studies of membrane and synaptic ionic channels are difficult to pursue in the intact slice. We have attempted to develop a semi-isolated neuron preparation using adult animals that would enable us to investigate the properties of single channels using patch-clamp methods.

Enzymatic treatment and mechanical dissociation were used to obtain a preparation of totally and partially isolated neurons from adult, guinea-pig hippocampal slices. Partially isolated cells consisted of exposed somata and dendritic processes extending from fragments of the original slice. Cell types most often exposed were small pyramidal cells and granule cells. The patch-clamp technique for single-channel recording was used to measure currents through single GABA-activated channels in cells either totally or partially isolated. Patches of membrane were excised after seals of > 10 gigaohms were formed. Both bath and pipette solutions contained predominantly choline chloride so that the chloride equilibrium potential could be known with certainty, and also so that currents from other channels would not contribute to the current record. The extracellular surface of the membrane patch was exposed to GABA in a range of concentrations from 0.1 to 1.0 μM. Current records from patches of membrane with evidence for the presence of only a single channel were analyzed for open channel current level and open and closed times.

Values of the single channel conductance were in the range of 19-24 pS, which is comparable to values obtained from other preparations. Histograms of the channel open time could be fit by single exponential curves with time constants in the range of 0.5 to 2.0 msec. Closed time histograms were better fit with multiple exponentials.

Our results will be used to test various kinetic models for the gating of GABA-activated channels. Using this preparation, it now appears feasible to investigate the biophysical properties of ionic channels from adult mammalian neurons. (Supported by the McKnight Foundation and NIH grants NS11535 and NS15772).

345.8 EFFECTS OF LITHIUM IONS ON LEAK AND VOLTAGE-GATED OUTWARD CURRENTS IN PERFUSED SNAIL NEURONS. D. Junge. Sch. of Dent. and Dept. of Physiol., Univ. of Calif., Los Angeles, CA 90024.

Lithium injection has been shown to increase the resting potassium permeability of snail neurons (Partridge, L.D. and Thomas, R.C., J. Physiol. 254:551, 1976), and external application of lithium may depolarize these neurons (Gardner, D.R. and Kerkut, G.A., Comp. Biochem. Physiol. 25:33, 1968, Fig. 2), presumably by blocking the electrogenic sodium pump. I have studied the effects of injection or external application of lithium on isolated, perfused ganglion cells from Helix aspersa (mainly cell RP-1), using the voltage-clamp method of Lee et al. (Lee, K.S., Akaike, N. and Brown, A.M., J. gen Physiol. 71:489, 1978). Series resistance was compensated electronically but leak currents were not. The normal external solution contained 75 mM NaCl, 5 mM KCl, 10 mM $CaCl_2$, 15 mM $MgCl_2$, and 5 mM tris-Cl (pH 7.5). In Li-containing solutions, Na was replaced with Li on an equimolar basis. The internal solution contained 105 mM K-aspartate, 3 mM EGTA, and 5 mM HEPES (pH 7.3). Experiments were performed at 15-16°C to promote cell viability. The effect of replacement of 50% of the normal Na with Li was to slow and reduce the non-inactivating late outward currents (I) seen with 1/sec repetitive stimuli. The effect took 15 minutes to develop and the recovery in normal saline required 30 minutes. The experiment was conducted in K-free external solutions to block the electrogenic sodium pump. The leak current measured with hyperpolarizing commands was slightly reduced by the above application of 50% Li solution. The extrapolated leak currents for positive commands (I_1) were subtracted from the uncompensated currents to give the net active outward current ($I - I_1$). The compensated current was reduced 36% at +50 mV by treatment with 50% Li solution. In other experiments LiCl was iontophoretically injected into cells from a microelectrode filled with 2 M LiCl. The result of injection with a 20 nA current for 5 minutes was also to slow and reduce the outward currents, but in this case the leak was increased. The leak-compensated outward current was reduced 70% at +50 mV by Li-injection, and only recovered fully after 90 minutes. The increase in leakage current with injection of Li could account for the increased potassium conductance seen by Partridge and Thomas under the same conditions. Work is now underway to analyze the effects of Li on the kinetics of development of outward currents. (Supported by USPHS grant RR 05304.)

345.9 HYDROGEN ION CURRENTS IN SNAIL NEURONS. L. Byerly, R.W. Meech*, W. J. Moody*. Dept. of Biological Sciences, Univ. of South. Calif., Los Angeles, CA 90089.

Even when all the K^+ inside a snail neuron is replaced by Cs^+ or $Tris^+$, positive voltage pulses elicit large outward currents which show a voltage and time dependence similar to that of the delayed-rectifier K current. In the past this current was termed "non-specific" and assumed to be carried by Cs^+ or $Tris^+$. In experiments done on internally perfused Lymnaea neurons we have shown that these currents are mainly carried by H^+ and pass through channels that appear to be distinct from the channels previously identified in molluscan neurons. These H currents are probably the same as those recently identified by Thomas and Meech in Helix neurons (Nature 299:826).

These experiments were done with internal solutions that contained Cs aspartate, 100mM HEPES (or MES) and 5mM EGTA. The external solutions contained Tris chloride, 4mM $CaCl_2$, 4mM $MgCl_2$, and glucose (with 20mM MES for $pH_o = 6.4$). The diameter of the suction electrode opening was one third of the diameter of the cell, to give good control of pH_i. All experiments were done either with $pH_i = 5.9$, which blocks the Ca current, or after the Ca current had "washed out". The residual currents seen under these conditions are activated by positive voltage pulses. With $pH_i = 7.3$ & $pH_o = 7.4$ the residual currents are activated by pulses above 0mV. $T_{\frac{1}{2}}$, the time required for the residual current to reach half of the steady state value, is about 5ms at 30mV and decreases as the potential increases. The current shows very little inactivation over a 2s period.

Decreasing pH_i from 7.3 to 5.9 increases the size of the outward current and shifts both the I-V and $T_{\frac{1}{2}}$-V curves about 30mV in the negative direction. Decreasing pH_o from 7.4 to 6.4 shifts both the I-V and $T_{\frac{1}{2}}$-V curves about 50mV in the positive direction. The reversal potential (V_r) of these currents depends on pH_i and pH_o roughly as expected for a H-electrode. With $pH_i = 7.3$ V_r is about 50mV for $pH_o = 6.4$, -10mV for $pH_o = 7.4$, and below -40mV for $pH_o = 8.4$. With $pH_i = 5.9$ & $pH_o = 7.4$ $V_r \simeq -65$mV. Due to the shift of the I-V curve with pH_i and pH_o, steady-state inward H currents are not seen. V_r was determined from tail currents.

These H currents are very stable to internal perfusion, showing no washout over a period of hours. External Cd^{++} decreases the size and slows down the turn on of the H currents at a particular potential. External TEA is much less effective in blocking the H currents than it is in blocking the K currents in these cells. This work is supported by NS15341.

345.10 **CHANGES IN MEMBRANE CURRENT AND INTERNAL CALCIUM OF MOLLUSCAN NEURONS INDUCED BY INJECTED cAMP.** J.A.Connor and P.E.Hockberger. *Bell Laboratories, Murray Hill, NJ 07974. University of Illinois, Urbana, IL 61801.*

Responses to the injection of cAMP have been investigated in identifiable neurons from the abdominal ganglion of *Limax maximus* using voltage clamp and the calcium indicator dye, arsenazo III. Three basic types of response were found. The first type was found in two of the neurons routinely examined (soma diam. ~250 μm) as well as several others. Iontophoretic injection of cAMP, but not 5'AMP, cUMP, or other substances, induced an inward current. Amplitude of the current was graded with the injected amount of cAMP with values for the 250 μm cells in the 10 to 20 nA range for 50 sec injections of 50 to 100 nA. The duration of current flow outlasted the injection period by one to several minutes depending on the cell type and amount of nucleotide injected prior to a given test. During the period of current flow, the arsenazo absorbance (660-690 nm) increased, indicating a rise in cytoplasmic Ca ion. The signal was comparable to those resulting from the Ca influx during 0.5 to 1 sec voltage pulses to +20 mV. The dye signal slowly returned to baseline after the nucleotide induced current subsided. Hyperpolarizing the membrane during injections caused a small increase in both dye and current signals. Ion substitution experiments indicated that most of the current, perhaps more than 80%, is carried by Na ions. Both the nucleotide induced current and dye signal persisted in the presence of Cd ion (4 to 8 mM) even though the voltage gated entry of Ca was blocked. A second type of response was exhibited by the abdominal giant neuron, G cell, diameter ~300 μm. When injections were made while holding V_m at −40 mV or below, current and dye absorbance changes were negligible. At depolarized holding potentials, injection of cAMP gave a marked reduction of the outward current, due for the most part to suppression of a current with similar properties to the Ca-activated K current. Effects on the Ca-dye signal have varied (seasonally, we believe) from no effect of injection (summer-fall) to a 40 to 50% enhancement of the dye signal during voltage pulses (winter-spring). In a third type of neuron cAMP induced a Na current but had no direct effect on Ca (see Hockberger & Connor, these abstracts).

We thank Dr. A. Gelperin for specimens of Limax. A portion of this work not carried out at Bell Laboratories was funded by PHS NS 14186.

345.11 **CYCLIC AMP INJECTIONS INTO APLYSIA AND ARCHIDORIS NEURONS INDUCE SODIUM CURRENTS WITH UNIQUE ELECTRICAL AND PHARMACOLOGICAL PROPERTIES.** P.E.Hockberger and I.A.Connor. *Bell Laboratories, Murray Hill, NJ 07974 and University of Illinois, Urbana, IL 61801.*

Recently we reported that cyclic AMP injections into voltage clamped *Archidoris montereyensis* neurons resulted in reversible increases in membrane sodium current (Hockberger, P. and Connor, J. A., *Science*, 219:869, 1983). This effect has also been found using neurons from several other gastropod species, including *Aplysia californica*. We have examined the sodium current in greater detail using *Aplysia* cells R_2 and LP1 in addition to identified *Archidoris* neurons since the latter have been reported to be insensitive to TTX (Connor, J. A., *J. Physiol.*, 286:41, 1979). The identity of the charge-carrying species as Na was confirmed in two ways: (1) reversal potential of the current in low Na (20 mM) saline was approximately −10 mV; and (2) intracellular Na-sensitive microelectrodes recorded elevations in $[Na_i]$ during the current responses. In addition the magnitude of the Na-electrode response indicated that virtually all of the current could be accounted for as an influx in Na ions (n = 7, 17 injections).

The sodium current was relatively voltage-insensitive when examined using holding potentials between −20 and −100 mV. The current was evoked in cells R_2 and LP1 in the presence of bath-applied TTX (10^{-5}M), ouabain (5×10^{-4}M), or amiloride (1.5 mM). In both *Archidoris* and *Aplysia* neurons lithium substituted for sodium; however trisma (base), tetramethylammonium, or bis-tris propane substitution blocked the response. Thus, this cyclic nucleotide-induced sodium current did not appear to be mediated through sodium channels underlying the action potential upstroke (TTX-sensitive) or the negative slope resistance region (lithium-sensitive). It was not due to amiloride-sensitive Na-transport nor was it due to blockage of the Na-K pump (ouabain-sensitive). A net decrease in membrane chord conductance, measured by negative voltage pulses, was noted during current flow. The decrease was due to suppression of a TEA insensitive K conductance. The relative magnitudes of the resting membrane conductance, the observed conductance decrease, and the induced Na current are such to suggest that the I-V relationship for the Na current is very flat over the range where it is clearly measurable (−30 to −100 mV). A portion of this work not carried out at Bell Laboratories supported by PHS NS 15186.

345.PO PATCH-CLAMP RECORDINGS FROM HIPPOCAMPAL PYRAMIDAL NEURONS IN SYNAPTICALLY-INTACT SLICE FRAGMENTS. William H. Griffith, Richard Gray and Daniel Johnston. Neuroscience Program and Neurology Department, Baylor College of Medicine, Houston, Texas 77030.

A method has been developed that enables high-resolution "gigaseal" recordings to be made of whole cell and single-channel currents from neurons in semi-intact hippocampal slices. Furthermore, orthodromic excitatory synaptic inputs appear functional in this preparation.

Transverse slices (200-400 μm) from rat hippocampus were cut and maintained at 31-32° C in normal saline containing 3 mM K and 2 mM Ca. A variety of proteolytic enzymes have been utilized with variable success. The slices were treated with one or more of the enzymes for one hour in normal saline followed by washing. Experiments that record both the dendritic and somatic field potentials were used to assess the synaptic activity in the enzyme treated slices. Field potentials could be recorded from both these regions after enzyme treatment suggesting the functional integrity of at least some synaptic connections. Analysis of the field potentials also suggested that primarily the excitatory synaptic inputs remained intact, and these remaining inputs also displayed several forms of short- and long-term use-dependent plasticity.

Following enzyme treatment, single slices were transferred to a special recording chamber and viewed under 500X magnification (Hoffman Modulation Optics). A small section of tissue, lateral to stratum pyramidale, was excised to expose the pyramidal cells. This procedure was performed using a small piece of razor blade and requires a minimal duration of enzyme treatment in order to split the slice at the cell body layer. Exposed cells were patch clamped using standard techniques (Hamill et al., Pflugers Arch., 1981, 391:85) with a patch-clamp constructed after the Yale design (Mark V, 1 gigaohm headstage). The internal pipette solution contained (mM): 150 KCl or K-Aspartate, 1 $MgCl_2$, 1 EGTA, 10 HEPES and pH 7.3 with KOH.

Voltage-dependent sodium and potassium currents have been recorded using the whole-cell clamp configuration. In addition, single-channel currents, usually from cell attached patches, were also recorded. We believe that this method of enzyme treatment and patch clamping has advantages over totally dissociated pyramidal neurons when studying the synaptic and/or integrative properties of the slice. (Supported by the McKnight Foundation and NIH grants NS07182, NS11535 and NS15772)

AXOPLASMIC TRANSPORT III

346.1 ANTEROGRADE, RETROGRADE, AND TRANSNEURONAL TRANSPORT OF LECTINS. R.H. Fabian* and J.D. Coulter. Dept. Neurology and Marine Biomed. Inst., U. Tex. Med. Branch, Galveston, TX 77550.

Lectins are a class of proteins which bind to specific carbohydrate containing sites on cell membranes. Several lectins are known to be axonally transported and one lectin, wheat germ agglutinin, after anterograde transport, undergoes transneuronal transport from axonal endings into adjacent neurons (Ruda and Coulter, Brain Res. 249: 237-246, 1982). We compared seven lectins as to their properties in axonal transport: wheat germ agglutinin (WGA), concanavalin A (Con A), pisum sativum agglutinin (PSA), lens culinaris agglutinin (LCA), soybean agglutinin (SBA), ulex europaeus agglutinin (UEA), and peanut agglutinin (PNA). Anesthetized rats were injected with a 1% solution of lectin (10-50 ul) in various well defined neuronal pathways. These included subcutaneous injections in the vibrissal region for anterograde transport in trigeminal pathways and retrograde transport to facial motoneurons. Intraocular injections were used to examine anterograde transport in retino-geniculo-tectal pathways. After 24-72 hours, serial frozen sections were cut and stained by immunocytochemistry (PAP method) to localize the transported lectin. In some animals, different lectins were injected on either side to enable comparison between different lectins. Retrograde transport occurred with all lectins except PNA and UEA. WGA labeled the largest number of neurons, followed, in order, by Con A, LCA, and PSA, and SBA. Lectins with sugar specifities for N-acetyl-D-glucosamine (WGA), for D-glucose and D-mannose (Con A, LCA and PSA) and for N-acetyl-D-galactosamine (SBA) were retrogradely transported whereas the two lectins binding to L-fucose (UEA) and D-galactose, N-acetyl-D-galactosamine (PNA) were not. WGA, Con A, LCA and PSA were transported *anterogradely and in both the* trigeminal and retino-geniculo-tectal systems. Densest labeling occurred with WGA followed by Con A, LCA and PSA. All lectins transported in the anterograde direction were observed to lead to labeling of neuronal perikarya in the terminal fields of the trigeminal and retinotectal systems. Transneuronal transport was most evident with WGA, and less prominent with the other lectins, (Con A, LCA, PSA). The results suggest that lectins with different carbohydrate specificities (WGA versus Con A) are transported both antero gradely, retrogradely and transneuronally. Further, lectins with similar sugar specificities (e.g. Con A, LCA, PSA) show similar properties in axonal transport in the systems studied here. Supported by NS12481 and 11255.

346.2 SLOW AXONAL TRANSPORT OF NEUROFILAMENT PROTEINS IN ALUMINUM INTOXICATION. J. C. Troncoso*, J. W. Griffin, P. N. Hoffman, K. M. Hess-Kozlow*, J. R. Blum* and D. L. Price. Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Intrathecal administration of aluminum salts ($AlCl_3$) in the rabbit induces accumulation of neurofilaments in central nervous system neurons. In motor neurons (MN), these accumulations first appear in the proximal axon and subsequently in the perikarya (Troncoso, J.C. et al., Ann. Neurol., 12:278-283, 1982). Since neurofilaments are synthesized in the cell body prior to being transported distally via slow axonal transport, the spatial pattern of neurofilament accumulation following aluminum intoxication suggested a defect in neurofilament transport. Such a mechanism for the formation of neurofilament swellings is not without precedent: neurofilamentous swellings occurring in the proximal axon in β,β'-iminodipropionitrile intoxication result from a selective impairment of neurofilament transport (Griffin, J.W. et al., Science, 202:633-635, 1978).

In order to test this hypothesis, the axonal transport of cytoskeletal proteins was examined in the lumbar MN of three-week old rabbits intoxicated by the intracisternal injection of $AlCl_3$ (100 μl, 1% w/v, pH 3.5). Five days later, MN were labeled by microinjection of [^{35}S] methionine into the lumbar spinal cord. In both intoxicated animals and controls, transport was analyzed at one, two, and three weeks after labeling. The distribution of transported proteins in sequential 3-mm segments of the sciatic nerve and roots was analyzed using SDS-PAGE and gel fluorography. In order to determine the distribution of radioactivity in individual proteins, gel bands were removed, dissolved, and their radioactivity measured. Transport *in control rabbits* was similar to that previously described in other mammalian species. The mean transport velocities for both the neurofilament triplet proteins and tubulin were approximately 25% less in the nerves of intoxicated animals as compared to controls. In addition, the ratio of neurofilament protein to tubulin in the nerves of control animals was 1.6 times greater than in the nerves of intoxicated animals. Thus, the transport of neurofilaments appeared to be reduced following intracisternal intoxication with $AlCl_3$. These results suggest that a defect in neurofilament transport may be important in the pathogenesis of neurofilamentous swellings induced by aluminum.

346.3 TRANSPORT OF NEUROFILAMENT POLYPEPTIDES IS ACCELERATED IN GIANT AXONAL NEUROPATHY. S. Monaco*, L. Autilio-Gambetti, R. Crane*, P. Gambetti. Institute of Pathology, CWRU, Cleveland, Ohio 44106.

2,5-Hexanedione, a γ-diketone hexacarbon, causes an axonopahy morphologically characterized by enlargements located in the preterminal regions of axons. These enlargements are sites of accumulation of neurofilaments (NF). The effect of 2,5-HD on slow axonal transport was investigated in rat primary optic pathway labeled by intraocular injection of ^{35}S-methionine. Slow axonal transport was analyzed in consecutive segments of the primary optic pathway, using one- and two-dimentional gel electrophoresis and fluorography. The transport rate of NF and that of two other polypeptides of 64 and 62 Kd normally migrating with the SCa was increased 2-3 times whereas those of tubulin and polypeptides of the SCb were unaffected. Ultrastructural examination showed a dramatical decrease of NF along the entire length of the optic axons proximal to the NF loaded enlargements. It is suggested that the increased transport rate of NF produces the longitudinal rearrangements of these structures that results in proximal decrease and distal accumulation of NF in the axons. Because of the kinetical separation of NF and tubulin, the 2,5-HD axon may be a useful model to study the structural associations among the polypeptides of the SCa. (Supported by NIH Grant NS 14509. Dr. Monaco is supported by a grant from the Standard Oil Company of Ohio.)

346.4 EFFECT OF ALUMINUM ON NEUROFILAMENT TRANSPORT AND MORPHOLOGY OF PERIPHERAL AXONS. A. Bizzi*, L. Autilio-Gambetti, R. Crane*, P. Gambetti. Institute of Pathology, CWRU, Cleveland, Ohio 44106.

Aluminum induces the accumulation of neurofilaments (NF) in the perikaryon and proximal processes of neurons, with the formation of neurofibrillary tangles (NFT). Impairment of the axonal transport of NF could be one of the mechanisms leading to this accumulation. We have investigated this possibility in the rabbit hypoglossal system.

Local administration of $AlCl_3$ produced NFT in 90-100% of the hypoglossal neurons. After labeling the hypoglossal nucleus with ^{35}S-methionine, polypeptides transported with the slow component of the axonal transport were studied by one- and two-dimensional gel electrophoresis and fluorography of consecutive segments of the hypoglossal nerve. Eighteen days after administration of the precursor, labeled NF polypeptides in control nerves had migrated up to 27 mm, whereas in the Al-treated animals their transport had stopped in the proximal 6-9 mm nerve segment. A polypeptide of 57000 MW was similarly affected whereas transport of tubulin as well as of polypeptides of the component b of the slow transport were apparently normal.

Morphological studies revealed that axons in the proximal 6-9 mm nerve segments were enlarged and loaded with NF whereas they were markedly reduced in size and lacked NF in the segments immediatly distal.

We conclude that administration of Al impairs the transport of NF and of a 57000 MW polypeptide within the proximal segment of the nerve, while translocation along the axon of NF that have already entered the slow transport system is not affected. This alteration in NF transport results in accumulation or lack of NF in adjacent segments of the same axon. (Supported by NIH Grant AG 00795.)

346.5 EVIDENCE FOR THE FAST TRANSPORT OF TUBULIN AND ACTIN IN MAMMALIAN SCIATIC NERVE by D.P. Stromska, Z. Iqbal, and S. Ochs, Dept. of Research, Mercy Hospital and Medical Center, Chicago, IL., 60616, Dept. of Neurology, Lakeside VA Hosp., Chicago, IL., 60611, and Dept. of Physiology, Indiana University School of Medicine, Indianapolis, IN., 46223

There has been controversey over the past several years as to whether or not the microtubule protein subunits, tubulin, and the microfilament subunits, actin, undergo fast axoplasmic transport. Initial evidence from sciatic nerve proteins labeled with ^{35}S-methionine and separated on one dimensional SDS-polyacrylamide gels suggested that there was fast transport of these two proteins, but the amount of labeled protein was small (Stromska, Iqbal, and Ochs, Soc. Neurosci. Abst., 5:63, 1979). Further evidence using two-dimensional electrophoresis methods was required for positive identification. Conventional methods for the two-dimensional electrophoresis of proteins had proven to be inadequate for the analysis of trace quantities of labeled proteins, particularly tubulin. A new method for 2-D electrophoresis has been developed which has improved the ability to separate and detect tubulin and other proteins from nerve without the loss of proteins that usually results from using the O'Farrell (1975) method of 2-D electrophoresis. Rat L5 dorsal root ganglia were injected with ^{3}H-leucine and periods of downflow allowed from 5 hr to 21 days. The sciatic nerves were removed, cleaned in the customary fashion, and cut into 5 mm segments, all at cold temperatures. The segments of nerve were then extracted with cold chloroform:methanol (2:1, v/v) until all of the lipid had been removed. This step was found to be essential for getting tubulin, actin and the neurofilament triplet into the gel for analysis. The segments were then dried and homogenized in 7 M urea, 5% 2-ME, 1% NP-40, and a 1:16 dilution of Pharmalytes pH 4-6.5. Three sequential segments were combined to make one sample. The proteins were analyzed by the new 2-D method (Stromska, submitted for publication) which employs a flat slab of agarose as the first dimension gel. Spots corresponding to tubulin and actin were cut out and radioactivity detected by scintillation counting. The patterns of downflow for tubulin and actin were identical to those observed for the overall pattern of radioactivity in the nerve. The amount of fast transported tubulin and actin are between 0.01 and 0.1% of the solubilized, labeled protein in the nerve. These results are consistent with the unitary hypothesis of axoplasmic transport and could support this model. The exact subunit species transported in the fast and slow phases remains to be determined. Supported by the Blum-Kovler Fellowship and PHS Grant NS08706-14.

346.6 RAPID AXONAL TRANSPORT OF TWO MOLECULAR FORMS OF Na, K-ATPASE IN THE OPTIC NERVE OF ALBINO RATS. S.C. Specht and K.J. Sweadner. Dept. of Pharmacology, Univ. Puerto Rico Sch. Med., San Juan PR 00936; Neurosurgical Research, Mass. General Hospital, Boston, MA 02114.

The Na,K-ATPase is a membrane-bound enzyme and is transported in the rapid phase of axonal transport. Two molecular forms of the catalytic subunit, which can be identified by their electrophoretic mobilities and affinities for cardiac glycosides, can be isolated from brain (Sweadner, 1979, J. Biol. Chem. 254:6060-6067). One of these, called α, is the only form present in cultured glia cells, whereas the larger form, α+, is the only form in axolemma.

When the isolated optic nerve of albino rats was incubated in a nutrient medium with ^{35}S-methionine, only the α form was synthesized by the sheath cells. To determine if neuronal cell bodies synthesize both forms, we examined the axonally transported proteins of the retinal ganglion cells. Proteins were labeled by intravitreal injection of ^{35}S-methionine and the labeled enzyme was subsequently purified from retina, optic nerve and lateral geniculate body/superior colliculus (LGN/SC). The predominant form was α+, but a small amount of α was also labeled.

To test whether the labeled α had been synthesized by glia in the vicinity of the nerve endings, using ^{35}S-methionine obtained by periaxonal diffusion from the eye or breakdown of axonally transported protein, we inhibited local protein synthesis by continuous intracisternal infusion of cycloheximide and unlabeled methionine. Inhibition of local protein synthesis did not affect the results. Intravitreal injections of either colchicine or vinblastine blocked the appearance of both forms of labeled enzyme equally. Time course studies gave no evidence of interconversion of the two forms in optic nerve or nerve endings.

Hence, the data indicate that while α+ is the predominant form of the catalytic subunit of the Na,K-ATPase, small amounts of α are also made and axonally transported by the retinal ganglion cells. (NS-07464 to SCS and NS-18233 to KJS.)

346.7 ROUTING OF TRANSMITTER AND MODULATION OF FAST AXONAL TRANSPORT FOLLOWING TRANSECTION OF ONE BRANCH OF THE BIFURCATE AXON OF AN IDENTIFIED APLYSIA NEURON. J.M. Aletta and D.J. Goldberg, Dept. Pharmacol. and Ctr. Neurobiol. & Behav. Columbia U., New York, N.Y. 10032.

We are interested in determining how a neuron regulates the amount and composition of material conveyed to its synapses by fast axonal transport. Using an identified neuron with a bifurcate axon, we have been studying the way the cell body adjusts its export of transmitter following removal of some of its synapses by transection of one axonal branch. We now report that routing of transmitter is one such adjustment elicited by this removal.

Previous results from our laboratory have demonstrated a rapid and precise down regulation of serotonin transport following in vivo transection of one branch of the bifurcate axon of the giant cerebral neuron (Science 218:913, 1982). We have found that transport of ^{3}H-fucosyl glycoprotein, which normally is associated only with the serotonergic vesicle in this axon, decreases to the same extent as transport of ^{3}H-serotonin. The glycoprotein down regulation occurs much more rapidly, however, probably because ^{3}H-fucose labels only newly synthesized vesicles, whereas ^{3}H-serotonin labels pre-existing vesicles as well. The transport of ^{3}H-fucosyl glycoprotein does not remain diminished: by 3 days after the transection it has begun to rise and -preliminarily- has surpassed the normal level within 2 weeks. Because transport of ^{3}H-serotonin is stably diminished during this period, this result may indicate that ^{3}H-fucosyl glycoprotein has undergone a redistribution into organelles not normally labelled and transported in intact neurons.

We have also found a clear instance of routing of transported material. Instead of cutting one branch close (~1mm) to the bifurcation, as done previously, we transected both branches far (~10mm) from the bifurcation, expecting the branches to continue to be capable of transporting normal amounts of transmitter. Indeed, 12 hours after the transections, when there is as yet no significant down regulation of transmitter, transport of ^{3}H-serotonin into both branches was normal. However, when only one branch was cut distally and the other left intact, assays of transport at 12 hours showed that much of the ^{3}H-serotonin that would ordinarily go into the transected branch was diverted into the intact branch. Thus, transmitter is directed away from an axonal branch lacking its synaptic endings and into an intact branch even when the transected branch is physically capable of transporting its normal complement of transmitter.

346.8 ALTERATIONS IN NOREPINEPHRINE METABOLISM IN RESPONSE TO AXONAL INJURY OF LOCUS COERULEUS NEURONS. B. E. Levin, Neurology Serv., VA Med. Ctr., E. Orange, NJ 07019, and Dept. of Neurosciences, NJ Med. Sch., Newark, NJ 07009

Adult male Sprague-Dawley rats were injected in the right cerebral hemisphere with the neurotoxin 6-hydroxydopamine (6-OHDA) at a site which interrupted the noradrenergic axons ascending from the locus coeruleus (LC). Distal to the injection site ("posterior cortex"), levels of norepinephrine (NE), dopamine-β-hydroxylase (DβH) and tyrosine hydroxylase (TH) fell to 39-42% of control levels ipsilateral to the lesion over the first 25 days, while contralateral levels fell to 32-73% of control during this time. These changes were paralleled by a 63% decrease in the high affinity uptake of {^{3}H} NE in the ipsilateral posterior cortex at 12 d after the lesion. Both ipsilateral and contralateral levels of NE and DβH fell in the LC during this time, while LC TH showed variable increases and decreases in activity. At 3 mo after right cortical 6-OHDA injections, posterior cortical levels of NE, DβH and TH, as well as the high affinity uptake of {^{3}H} NE, had returned to control levels suggesting that some type of regeneration or axonal sprouting had occurred. Axonal transport of DβH and TH was assessed by measuring the accumulation of enzyme activity proximal to a 6-OHDA lesion made in the more caudal portion of these same LC axons. Transport of DβH fell to 7%-40% of control from 2 d to 24 d and rose to 160% of control by 3 mo after the lesion. TH transport was decreased to only 61% of control only at 24 d and returned to control levels by 3 mo. These studies document that there is independent regulation of the metabolism of the NE synthetic enzymes, DβH and TH, during the degeneration and subsequent regeneration or collateral sprouting of injured distal axons of LC noradrenergic neurons.

346.9 THE ORIGIN OF THE AXOPLASMIC RETICULUM WITHIN THE AXON HILLOCK. James D. Lindsey and Mark H. Ellisman. Laboratory for Neurocytology, Department of Neurosciences, University of California at San Diego, La Jolla, CA 92093.

The axoplasmic reticulum (AR) is an extensive system of anastomotic tubules that extends down axons. To investigate the origin of this structure, spinal ganglia from Rana catesbiana were fixed in 2% osmium tetroxide for 1 hr. and then impregnated in the same solution at 37°C for 36 hrs. Following impregnation, some of the ganglia were treated with Walton's lead aspartate for 1 hr. All ganglia were subsequently dehydrated and then embedded in Epon-Araldite. Sections varying in thickness from 80 to 700nm were studied stereoscopically. Serial sections 170nm thick were also prepared and micrograph series through regions containing axon hillocks were taken. Enlargements of these micrographs were reproduced onto transparant acetate sheets allowing structures cut apart during sectioning to be precisely superpositioned during reconstructive analysis. In moderately impregnated neurons, AR and the cis elements of the Golgi apparatus were filled with an electron dense deposit. The rough endoplasmic reticulum (RER) displayed a light granular staining. Discrete axonal cisternae (with the exception of multivesicular bodies that occasionally contained impregnated vesicles) plus the trans elements of the Golgi apparatus were not impregnated nor displayed granular staining like RER. Thus the various membranous structures found within the axon hillock and cell soma were easily identified even though only a small fragment might appear in any given section. RER often extends into the proximal axon for approximately 10μm ,in these neurons, thus defining the limits of the axon hillock.

Within the distal hillock region, AR arose directly from sparse elements of RER. The AR was occasionally accompanied by impregnated tubules also arising from RER but only extending down the axon for a few microns before terminating blindly. The proximal hillock region contained many impregnated tubules similar to those just described except that they were usually shorter. Occasionally these short tubules appeared to rejoin the RER. Impregnated tubules arising in this region were not observed to join with AR. In the distal hillock, small unimpregnated discrete cisternae were usually seen closely associated with AR. In contrast, within the proximal hillock region, the small unimpregnated discrete cisternae were most often closely associated with RER elements. Images suggesting continuities between these small discrete cisternae and either AR or RER were not seen. (Supported by grants from NIH #NS14718, MDAA, and NMSS to MHE.)

346.10 SEMI-AUTOMATED, COMPUTER ANALYSIS OF ORGANELLE TRANSLOCATION IN AXONS, A.C. Breuer, R.E. Dayhoff,* R.S. Ledley,* and A.W. Dudley, Jr.* Cleveland Clinic Foundation, Cleveland, Ohio; Georgetown University Medical Center, Washington, D.C.

The mechanism of fast axonal transport, the process by which substances made in the cell body are moved long distances along nerve cell axons, remains a major unsolved problem in biomedicine. The discovery of the video-enhanced contrast method (Allen R.D., Allen N.S., Travis J.L. Cell Motility 1: 291-302, 1981) coupled with differential interference contrast optics makes possible the detection of intra-axonal organelle traffic in unprecedented detail. Thus far relatively few measurements of this motion behavior have been made. Recently, the image output of a Zeiss Axiomat microscope and Hamamatsu video system have been coupled to a TEXAC whole-picture image analysis computer (Ledley R.S., Kulkarni Y.G., Park C.M., Shiu M.R., Rotolo L.S. Proc IEEE Computer Society Conference on Pattern Recognition and Image Processing, Chicago, pp 396-410, 1978) where analogue data are digitized, image processed, tracked, and graphically displayed. Images are recorded on a high speed 160 megabyte Winchester digital disc, and then enhanced. Enhancement procedures include video frame averaging for noise reduction, whole picture subtraction which eliminates all image elements which have not moved from one frame to the next, and thresholding. Organelle size, mean gray level and X-Y pixel coordinates are used to identify structures to be tracked. Tracking is carried out interactively by an operator who positions a cursor rectangle over an organelle of interest using joystick control. When tracking is completed, calculation and display of variables such as distances travelled, instantaneous (0.3 sec) velocities and accelerations in multiple graphic formats is rapidly accomplished. Hard copy output of graphic information is readily obtained from a Tektronics Hard Copy Screen Printer. The advantages of this system are: 1) its high spatial and temporal resolution; 2) its capability of providing a large amount of information in a relatively short time, and 3) the modifiability inherent in a programmable tool. The system is being used to define multiple variables of fast axonal transport in cultured neurons, marine invertebrate giant axons, and normal and diseased human nerve. It may yield insight into several lethal human neurologic diseases such as Alzheimer's Disease and Amyotrophic Lateral Sclerosis.

346.11 REACTIVATION OF THE FAST AXONAL TRANSPORT MECHANISM IN GLYCEROL-PRESERVED SQUID AXONS. Alan J. Hodge* and William J. Adelman, Jr.. Lab. of Biophysics, NINCDS, NIH, Marine Biological Laboratory, Woods Hole, MA 02543.

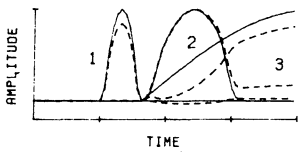
The structural integrity of the mechanism(s) underlying fast axonal transport appears to have been preserved in squid axons by a procedure comparable to that commonly used in preparing glycerinated muscle. 4-6 cm lengths of medial giant axons with associated small fibers and attached stellate ganglion were preserved by immersion in a cold 50% glycerol solution containing 5 mM EGTA, 5 mM Mg++ and 20 mM tris buffer, incubation for several days at ∿ 4°C followed by long-term freezer storage at ∿ -5°C. After several weeks, carefully excised lengths (5-10 mm) of these preparations were incubated at ∿ 4°C in an isotonic buffered isethionate solution containing 0.1 mM Ca^{++}, 1 mM Mg^{++}, and 3 mM ATP and other components including taurine and sucrose. The movement of particles in both orthograde and retrograde directions observed after several hours of incubation were indistinguishable from the fast axonal transport seen in freshly dissected squid axons using video-enhanced differential interference microscopy. It appears, therefore, that the glycerol-preserved axon may prove to be a valuable model system in defining the functional and structural aspects of fast axonal transport.

EVOKED POTENTIALS AND EEG

347.1 PRINCIPAL COMPONENT ANALYSIS OF ELECTROPHYSIOLOGICAL SIGNALS: SIMULATION STUDIES. C.C. Wood and G. McCarthy*. VA Medical Center, West Haven, CT 06516 and Yale U., New Haven, CT 06520.

Electrophysiological signals and other neurobiological time series pose difficult analytic problems because of their inherent statistical dependencies. Principal component analysis (PCA) is widely used to decompose such correlated data into small sets of statistically independent components for analysis of experimental effects. We used simulated components typical of recent event-related potential (ERP) studies to investigate PCA's ability to reconstruct component waveshapes and to allocate variance correctly across components. The simulated ERPs consisted of 800 randomly weighted combinations of three 64-point components, corresponding to a 2 X 2 X 2 design with 100 subjects. A large main effect of one experimental treatment was introduced on component 2 with no other main effects or interactions. Co-variance PCAs, Varimax rotations, and univariate ANOVAs were performed on each of 100 such simulations. The waveforms of the derived components (dotted) corresponded reasonably well to those of the original components (solid). However, comparison of component scores with the original random weights indicated that PCA incorrectly allocated variance across components: components 2 and 3 accounted for approximately 37% and 50% of the original variance, respectively, but the corresponding derived components accounted for 46% and 41%. In ANOVAs on the original weights, only the main effect for component 2 was significant, but that effect was significant for both components 2 and 3 in ANOVAs on the component scores. While 6 of the 100 ANOVAs on the component 3 weights were significant at the 5% level, 74 of the 100 ANOVAs on the corresponding component scores exceeded that level. Thus, the Type I error rate for component 3 was inflated from 5% to 74% due to PCA's incorrect allocation of variance across components.

These results are limited in scope and should not be overgeneralized. Nevertheless, they indicate that PCA can distribute variance incorrectly across components, resulting in serious misinterpretation of treatment effects. Comprehensive simulation studies are needed to assess the extent of this problem across systematic variations in the number, shape, overlap, and variance of simulated components, and in the specific form of PCA employed. Pending such studies, we believe that results based solely on PCA should be interpreted cautiously.



347.2 TWO DIPOLE GENERATORS IN HIPPOCAMPAL THETA RHYTHM: EXPERIMENTS AND MODEL. L. Stan Leung, Department of Psychology, University of Western Ontario, London, Ontario, Canada N6A 5C2.

A gradual phase shift of the theta rhythm of 180° over 400 um was found in stratum radiatum of hippocampal CA1 region in the rat during undrugged walking and active sleep. (Winson, Electroenceph. clin. Neurophysiol. 36: 291, 1974). Under curare or urethane, an abrupt phase shift was found in proximal stratum radiatum. Previous and recent models (e.g. Holsheimer et al. Brain Res. 235: 31, 1982) have not accounted for the gradual phase shift. In the present model, two standing dipole generators of different potential and source-sink profiles in CA1 region were assumed, roughly corresponding to the early excitatory (E-) postsynaptic potential (PSP) and the late inhibitory (I-) PSP field in CA1 following association fiber stimulation (Leung, Brain Res. 176: 49, 1979). By assuming each dipole was driven by a theta frequency driving function with a fixed phase shift between the driving functions, the gradual phase shift over 400 um could be predicted.

In chronically implanted rats, electrodes placed in mid-stratum-radiatum gave 120°-160° phase with respect to the theta recorded at the alveus. Eserine increased this phase towards 180° and atropine/scopolamine towards 90°. During immobility after ether or urethane, the theta phase was 180° and this theta could be abolished by atropine (Vanderwolf et al., The Hippocampus, Vol. 2, p. 101, 1975). A second-by-second spectral analysis of the theta rhythm and radiatum phase during active sleep indicates that the phase was about 100°-180°, but a significant positive correlation of phase with peak theta frequency was found (i.e. phase was closer to 180° at high theta frequency).

The significance of this study are: (1) The gradual theta phase shift is the result of two standing dipole fields being driven out of phase. (2) The two dipole fields are consistent with an atropine-sensitive and an atropine-resistant theta. (3) During normal behaviors (e.g. active sleep), both fields appear to be present but their amplitudes may vary spontaneously and dynamically. To my knowledge, this is the first time that the gradual phase shift in radiatum has been integrated with the pharmacological properties of the theta rhythm.

347.3 A LAMINAR ANALYSIS OF THE CORTICAL FLASH VISUAL EVOKED POTENTIAL IN THE MONKEY. M.A. Kraut*, J. C. Arezzo and H. G. Vaughan, Jr. Depts. of Neuroscience and Neurology, Albert Einstein Coll. of Med., Bronx, N. Y. 10461.

Evoked potentials are of greater experimental and clinical utility when the neural elements and physiological processes responsible for waveform genesis are identified. To this end we have examined the intracortical laminar patterns of flash visual evoked potentials (VEP), multiple unit activity (MUA) and current source density (CSD) within area 17 of the unanesthetized monkey. Data were collected from 50 depth passes in 3 monkeys (M. fascicularis) using multicontact electrodes with impedances of approximately 300 Kohms and interelectrode spacings of 75 or 100 um.

The earliest component detectable in the VEP is a small positivity peaking at 18 msecs (P18). This component is invariant in amplitude and polarity within the cortex and is not correlated with either MUA or CSD activity, indicating its subcortical origin. P18 is followed by a N24-P28 complex that inverts in lower lamina III or in lamina IVA. The onset of N24 is associated with the earliest detectable increase in MUA and with a small current sink within lamina III. The corresponding source extends for approximately 200 um below the level of VEP polarity inversion. A large negativity is present throughout the upper laminae which differentiates into at least two sub-peaks within the upper portion of lamina IV (N39 and N44). Each of these components inverts at the same depth within lamina IVC. The CSD in this region is characterized by a large current sink sandwiched between coincident sources extending above and below the level of VEP polarity inversion. A series of oscillatory potentials having a period of 5-6 msecs, are present on the falling edge of N40 and are associated with discrete bursts of MUA throughout lamina IV. The onset of MUA within lamina IVC follows the earliest MUA within lamina III by about 8 msecs. Additional VEP components are N55, P75 and P120. While neither N55 nor P120 are correlated with specific MUA, N55 is associated with complex laminar patterns of current flow. P75 is associated with diminished MUA and CSD having a spatial distribution suggesting the hypothesis that this component reflects inhibitory activity in cellular elements spatially coincident with those responsible for the generation of N40.

Analysis of these laminar profiles provides a basis for testing hypotheses regarding the cellular elements that underlie specific VEP components. Our data exhibit a considerably more complex pattern of intracortical activity in the unanesthetized monkey stimulated by light than that observed in anesthetized animals following electrical stimulation of the visual pathways.

Supported in part by NIH NRSAs NS 07183, MH 15788, and by MH 06723 from the USPHS.

347.4 POISSON-ELECTRICAL STIMULATION OF AN AXONAL BUNDLE M.D. Goldfinger,W.Kuo*,&K.P.Zimmermann. Dept.Biology&Sch.Medicine, Univ.Missouri-Kansas City,MO 64110; Electrical&Computer Engineering Univ. Missouri-Columbia, MO 65211

Analysis of evoked electrical activity needs to assess possible contributions by both linear and nonlinear mechanisms,particularly for complex waveforms elicited by synchronous activity of groups of neurons.The present work considers the case of the compound action potential(CAP) of an axonal bundle. Linear and nonlinear aspects are assessed by analysis of the CAPs elicited by a Poisson stimulus sequence.

A 4-cm.length of sciatic nerve is excised from small pithed grassfrogs and placed in a chamber containing Ringer solution.One nerve end is drawn up into a suction recording electrode;the other end lies across a pair of AgCl wires through which short(0.1 ms) rectangular isolated stimuli are applied.Stimulus amplitude and conduction distance are great enough to demonstrate activation of 3 fiber groups(2-5-fold greatest difference in conduction velocity).

The Poisson stimulation process is described for sequences of >1000 pulses.A stack of 2011 values obtained from nuclear disintegration is used by a microprocessor to drive the nerve stimulator. The interstimulus interval distribution consists of a deadtime (.36 -.64 ms),a finite rising limb(usually <1 ms),and a falling limb well-fit(r>.96)by 1 exponential.Mean stimulus rates (L=1/mean interval)used in respective runs ranged from 92-150/sec;sequence deadtime varies inversely with L.Coefficient of variation of the entire distribution(deadtime-corrected)ranged from .88-.99. The Expectation Density(ie,autocorrelation)consists of the deadtime, finite rising limb,and maintained noisy-envelope plateau.

For each run,a train of Poisson stimuli at a given L was applied to the nerve continuously for appr.20 sec;a single CAP was elicited immediately before and after Poisson stimulation.Taped data are digitized at 11 us and stored on disk for subsequent analysis of the first 182 ms of the run.

With the onset of Poisson stimulation successive CAPs show much variability as short interstimulus intervals occur during axonal refractoriness.Using the analytical method of Krausz(1),the 1st-order Wiener kernel is computed and compared to the CAP elicited by 1 stimulus pulse immediately before and after Poisson stimulation.This kernel does not fully represent either single-trial CAP: only the largest-fiber component obtains.This result implies that nonlinear intrinsic mechanisms(not due to the stimulus sequence) contribute to the single-trial CAP,and need to be included for its complete reconstruction.

(1)H.I.Krausz,Biol.Cyber.19:217-230,1975.

Supported in part by Weldon Springs Endowment Fund, University of Missouri-Kansas City

347.5 SPATIAL STRUCTURE OF CORTICAL EEG: SYNCHRONY OF SMALL POPULATIONS CAN BE MEASURED BY COHERENCE AS FUNCTION OF DISTANCE. T.H.Bullock G.D.Lange and M.C.McClune*. Neurobiology Unit, Scripps Inst. Oceanog. and Dept. Neurosciences, U.C.S.D., La Jolla, CA 92093.

Ongoing electrical activity in the quiet, awake vertebrate, recorded from optic tectum or forebrain is similar in all classes from fish to mammals in having a broad a.c. (>0.5 Hz) power spectrum peaking between 5-15 Hz and falling >30 dB below mean power by ca 50 Hz - irrespective of brain size, development of cortex, electrode type, etc. Little is known of the fine structure of the field. It is generally believed to be the vector sum of many generators, chiefly membranes of parts of cells with a similar power spectrum on average. This would mean (a) spikes and their synchrony are unimportant for EEG, (b) proportion of congruent generators and degree of their synchrony of slow potentials are important, (c) there may be a complex field structure in space. Goals of this study are to quantify an estimate of synchrony and discover its properties in rabbit, guinea pig and rat, allowing eventual comparisons of brain regions and states and taxa.

EEG was recorded in alert, drowsy or seizure states by an array of 5+ metal semimicroelectrodes implanted extradurally, subdurally or intracortically, with a common reference shown to have little activity, usually yoked frontal sinuses. Taped data was low-passed to 48 Hz, digitized at 160/s, analyzed in epochs of 3.2 s by computing power spectra for each channel, coherence for each pair of channels at 1.25 Hz intervals, and 95% confidence lines. Coherence squared (COH), phase difference and log power (as dB from mean) were plotted against log frequency. Phase was consistently close to zero. Power and phase provide controls against some forms of meaningless coherence. Internal controls against excessive contribution from either independent or common mode noise were also provided by the agreement between different pairs of channels at the same electrode distance and the typically systematic decline in COH with distance.

Mean COH over the range 1-40 Hz usually shows no consistent peaks or valleys but a plateau, often gradually falling with frequency or falling rapidly from a maximum <4 Hz to a plateau above 8 Hz. Electrode separations of 0.5 mm often show COH significantly <1.0; 0.5 COH is typically at 3-5 mm for the low frequencies (1-6 Hz); at 8-16 mm it is barely distinguishable from COH of independent noise sources; the decline with log distance is more sigmoid than linear. These generalizations are based on agreement of means of 15-40 epochs but there are "odd-ball" samples. Midseizure COH is not specially high for small nor low for large distances, but bunched at ca 0.7 in the band of maxima, 12-16 Hz, in spite of frequent broad spikes synchronous in all channels. (Aided by NIH and NSF grants to THB.)

347.6 A COMPARISON OF MONOPOLAR AND BIPOLAR VISUAL EVOKED RESPONSES IN THE RHESUS. Fred H. Previc*, David L. Schafer*, and James A. Chambers* (SPON: B. Brooks). Life Sci. Div., Technology Incorporated, P.O. Box 32644, San Antonio, TX 78216.

According to several models, the visual evoked response (VER) is believed to be generated by dipoles oriented radially towards the cortical surface. By recording bipolarly (i.e., differentially) on opposite sides of the presumed dipoles, it was hypothesized that visual activity generated between the two leads would be enhanced whereas background EEG activity and visual activity occurring outside the region of the electrode would be diminished. Thus, a high degree of topographical resolution and a high signal-to-noise ratio should be associated with the bipolar VER.

Monopolar and bipolar VERs were recorded from a rhesus monkey under barbiturate anesthesia in response to grating-appearance and grating-counterphase. The stimuli were square-wave gratings which ranged in fundamental spatial frequency from 1.0 to 5.7 cycles/deg, and which possessed a contrast and luminance equal to 0.70 and 30 cd/m^2, respectively. The gratings were viewed by the right eye at a distance of 1 m. Bipolar VERs reflected activity recorded between the shallow and deep tips (3-mm separation) of a teflon-insulated stainless-steel electrode embedded in the left foveal projection region of area 17, whereas monopolar VERs were recorded between the shallow tip (resting on the dura) and an ear reference. In contrast to the more complex monopolar waveform, bipolar VERs elicited by the appearance phase of the grating were composed of a single positive component which peaked at approximately 100 msec and which was generated in the vicinity of the shallow lead. Bipolar recordings were also characterized by a much greater signal-to-noise ratio (which was largely a consequence of a marked reduction in the amplitude of the background EEG) and a more localized genesis (3 deg or less). The two recordings proved similar in terms of spatial frequency responsiveness, however, with both peaking between 2.0 and 4.0 cycles/deg. Finally, the bipolar VER was shown to be relatively insensitive to the anesthesia state of the animal, except at levels at which pronounced EEG spindling was evident.

The results demonstrate that the bipolar VER is useful whenever a highly localized and reliable electrophysiological assessment of visual function is required. The results are also relevant as to the understanding of the origins of various VER components, the sources of VER variability, and the effect of barbiturate anesthesia on cortical VERs.

Supported by Contract F33615-80-0610 from the USAF School of Aerospace Medicine, Brooks AFB, TX. Work was conducted at the Vulnerability Assessment Branch, USAF School of Aerospace Medicine.

347.7 AN ANIMAL MODEL FOR STUDYING FAR-FIELD SOMATOSENSORY EVOKED POTENTIALS DURING PERIPHERAL NERVE REGENERATION. D.D. Roscoe, M.W. Keith, L.M. Fay*, K.C. Jackson*, and B. Smith*. Evoked Potential Lab., St. Luke's Hospital, Cleveland, Ohio 44104.

An animal model is presented that can be used to study acute and chronic nerve lesions by means of far-field somatosensory evoked potentials (FFSEPs).

Currently, few chronic animal models exist that can be used as a vehicle to study nerve lesions, repair and regeneration under controlled conditions. This abstract describes a novel model that will enable FFSEPs to be recorded during stimulation of a normal peripheral nerve, transection and repair of the same nerve, and axonal regeneration of the repaired nerve. In any chronic study involving serial acquisition of FFSEP data, it is essential that the stimuli producing the evoked responses are constant. If this were not the case, one could not rule out the possibility that any changes observed in the FFSEPs over time were in fact due to introducing different stimulus parameters during each experiment. The significant feature of this model lies in the method of delivering an electrical stimulus to the nerve under investigation. To accurately deliver this stimulus, a sophisticated four-channel, RF-controlled implantable stimulator with nerve cuff electrodes distal and proximal to the induced lesion site is employed. The implanted stimulator provides the means for non-invasively exciting the test nerve with accurate and consistent stimuli.

FFSEPs were recorded from needle electrodes placed in the scalp of a dog (active electrode 6 cm anterior, reference electrode 2 cm posterior to the external occipital protuberance). Amplification bandwidth was set at 150 to 3000 Hz with an analysis time of 20 msec and a total of 2000 repetitions per average. Stimuli were delivered to the test nerves via an implantable RF controlled 4.5 ma constant current stimulator by placing an antenna over the implant site and transmitting serial control data to the implant. Highly reproducible FFSEPs were obtained by stimulating the median and ulnar nerves (8 usec pulse width) at a level just proximal to the cubital fossa and at the level of the wrist (14 usec pulse width).

With this model in hand, we will address a specific orthopaedic surgical procedure (i.e., repair of a transected peripheral nerve) by directly comparing the histology of the regenerating nerve to FFSEPs obtained just prior to biopsy of the test nerve. We will endeavor to correlate various stages of FFSEP recovery (stimulating distal to the lesion site) to the histological profile of the regenerating nerve.

This work is supported by the Orthopaedic Research and Education Foundation, Chicago, Illinois.

347.8 EFFECTS OF PHYSOSTIGMINE ON SEPTO-HIPPOCAMPAL EVOKED FIELD POTENTIALS. M.K. Hettinger and L.P. Gonzalez. Alcohol and Drug Abuse Research and Training Program and Department of Physiology and Biophysics, University of Illinois at Chicago, Health Sciences Center, Chicago, IL 60612.

This study characterized the evoked field potentials elicited in the CA3 field of Sprague-Dawley rats by paired-pulse stimulation of the ventral medial septum before and after exposure to physostigmine. Bipolar stainless steel electrodes were stereotaxically implanted in the ventral portion of the medial septum and the CA3 field of the hippocampus of male Sprague-Dawley rats. After recovery from surgery, constant current stimulation was delivered to the medial septum as single pulses or as paired pulses, with an interstimulus interval (ISI) ranging from 20 ms to 140 ms. Responses to 32 stimulus presentations were recorded and averaged to produce an averaged evoked potential (AEP). The ISI was held constant during each series of 32 stimulations, but was varied in a semi-random fashion between series, such that one AEP was obtained at each ISI. Stimulus intensities were set such that amplitudes of the components of the evoked potentials were half of the maximum amplitude and ranged from 250 μA to 350 μA. The same procedure was followed after injections of 0.04 mg/kg and 0.10 mg/kg physostigmine.

Three components were consistently observed in response to each stimulus of the pair. These included a positive peak at 6 msec after stimulus onset, a negative peak at 10 msec, and a second positive peak at 20 msec. These components of the response to the second stimulus in the pulse pair were significantly reduced when the interval between paired stimuli was 80-100 msec.

Physostigmine did not significantly change the amplitudes of the first positive component or the negative component of the first response; however, the amplitude of the second positive component was decreased. The amplitudes of the negative component and the second positive component increased in response to the second stimulus of the pulse pair after physostigmine. The observed decrease in amplitude of the various components in the ISI range of 80-100 msec was not altered.

These results suggest that the cholinergic portion of the septo-hippocampal pathway may play a role in the modulation of other, more primary, mechanisms that underlie the generation of field potentials in the CA3 field.

[This work was supported in part by Grant No. PHS AA 7374-03 from NIAAA.]

347.9 *GENERATOR OF P300 IN THE CAT.* *T. O'Connor and A. Starr.* Department of Neurology, California College of Medicine, University of California, Irvine, CA 92717.

Intracranial recordings of long-latency evoked potentials were obtained from an animal model of the P300. Muscle-relaxed, artificially-respired cats were presented with a variant of the "oddball" paradigm in which a "rare" 4KHz stimulus was intermixed with a "frequent" 1KHz stimulus. A pupillary dilation served as a behavioral index to the rare tone. A fixed skull electrode near the vertex served as a control recording site while electrodes were advanced into the brain through chronically implanted cannulas.

At intracranial locations waveforms associated with the rare tone generally differed substantially from the frequent stimulus waveforms in that the former usually manifested components of greater amplitude. The P300, which is positive at the vertex and dura, appeared as a negative component 4-6 mm below the surface of the marginal gyrus. Increasing the probability of the rare stimulus decreased the amplitudes of both the intracranial negative component and control P300. In the hippocampus a component occurred with a latency about that of the P300 which phase reversed between the dorsal and ventral aspects of that structure. Ablation of several mm of the marginal gyrus bilaterally modified significantly the P300 recorded from the vertex without affecting pupillary dilation. These results suggest that the cat P300 originates, at least in part, from the cortex of the marginal gyrus.

Supported by NIH Grant #NS11876-08

347.10 *STABILITY OF THE VISUAL EVOKED POTENTIAL TO INTENSITY SERIES OF LIGHT FLASHES IN CAT.* P. M. Saxton and J. Siegel, Institute for Neuroscience and School of Life and Health Sciences, University of Delaware, Newark, DE 19711 and J. H. Lukas, U.S. Army Human Engineering Lab., Aberdeen, MD 21005.

The evoked potential (EP) from the cortex to an intensity series of light flashes has been shown in both human and cat to relate to individual behavior. In some individuals cortical responsiveness is inhibited to high intensity stimuli and results in negative EP slopes (reducers), while other individuals have positive slopes (augmenters). To develop an animal model to investigate the physiological basis for such cortical response characteristics it is necessary to ascertain the stability of the visual EP both within and between sessions.

Eight cats with chronically implanted electrodes over primary visual cortex were Flaxedilized to prevent head movement, pupils were dilated with Mydriacyl, and the nictitating membrane relaxed with Neosynephrine. A Grass photostimulator 15 cm from the cat's eyes delivered 50 flashes at a rate of 1/sec at each of 5 intensities. Three intensity ranges were used. The high range at 24-1394 lumens$\cdot m^{-2}\cdot sec^{-1}\cdot sr^{-1}$ was approximately 1 and 2 log units higher than the medium and low ranges, respectively. Visual EPs were averaged on line with an Apple microcomputer and the amplitudes of the first 4 major components were determined.

Each cat responded to the intensity series of light flashes with characteristic EPs which were stable in shape over Flaxedil sessions a month apart as well as between intensity series on the same day. However, quantitative measurement of baseline to peak or peak to peak amplitudes showed a degree of variability dependent on the range of intensities. The lowest range showed stable amplitudes and slopes which were highly positive. Cortical response increased to increasing intensity of stimuli. In the highest range cortical responsiveness often decreased (negative slopes) but the amplitudes were as variable within as between cats. In contrast the VEP amplitudes and slopes in the medium range of intensities were quite consistent, both between runs on the same day and between sessions a month apart. Since these slopes range from very positive to negative, the degree of cortical inhibition in individual cats can now be investigated in its relation to both behavior and neural mechanisms.

347.11 AGE RELATED CHANGES IN THE VISUAL EVOKED POTENTIAL OF FISCHER 344 RATS, M.L. Weber-Levine and S.M. Levine. Psychology Dept., Morehouse College, Atlanta, GA 30314.

As part of an investigation studying the use of the rat as an animal model for aging research, visual evoked potentials (VEP's) were recorded from 5 Young (Y)(Mean age-9mos.), 5 Middle Age*(M) (Mean age-17mos.) and 5 Old*(O)(Mean age-29mos.) Fischer 344 male rats. Bipolar recordings were obtained from chronic electrodes implanted in occipital cortex (2mm lateral to lambda). The rats pupils were dilated with atropine and the freely moving animal was placed in an opaque chamber in a darkened room with whitenoise background. A Grass PS22 Photic Stimulator strobe unit positioned 20cm above the cage provided a uniform flash. Stimulus intensities of low, medium and high (strobe settings 1,4, & 16 respectively) were presented each day for three consecutive days in a Latin Square paradigm giving 9 sessions, 3 at each intensity. Daily test sessions were preceeded by an adaptation period of the same intensity as the first test session for that day. All timing and data collection was controlled by a Digital PDP11V03 computer. VEP's were fed through Grass P511J amplifiers into an A/D converter sampling at 2K Hz for 500msec. and stored on floppy discs. The intertrial interval was 20msec. The average curves are composed of 50 trials at each intensity level. VEP's were averaged by day and intensity for each subject and analyzed for peak amplitude and latencies.

Overall mean latencies were longer for the O Ss than both the M and Y groups for early and later components(O N1-44msec.,M & Y N1-36msec.). Test-retest reliability was strong within subjects but considerable variability existed across subjects in the O group (37-63msec. for O vs. 36-37 & 34-39msec. for M & Y respectively for N1). N1 peak amplitude across days was largest in the Y group (181μV) while the M and O were smaller (172μV). While amplitudes were somewhat varied, possibly reflecting fluctuating arousal levels, the most striking effect was seen in the very late peak-to-peak complex (N195-240 to P240-280). This complex also decreased monotonically with age, while showing a consistant habituation effect across all subjects by day. Effects of intensity on amplitude showed a complex interaction for early and later components for the different age groups.

The latency trends are generally consistent with that found in the human life span VEP data. Complexity of the amplitude data mirrors the controversial findings seen in the human VEP literature. Overall the data appear to support the use of the rat VEP in the delineation of the neurophysiological aspects of the aging process.

*Aged animals were from the NIA retired breeder colony.
(Supported by NIA Grant #5R23AG01583)

347.12 PRINCIPAL COMPONENT ANALYSIS OF A MODEL P3 RESPONSE IN YOUNG AND AGED CATS. Jean Harrison and Jennifer Buchwald. Brain Res. Inst., Ment. Ret. Res. Ctr., Dept. Physiol., UCLA Med. Ctr., LA, CA 90024.

The P3 potential is an endogenous positive potential recorded from the human scalp with a latency of 300 msec or more. Such potentials are produced by unexpected stimuli within a sequence of expected stimuli of the same modality or by the omission of an expected stimulus. The P3 has been associated with sequential processing and short-term memory functions and has been shown to be abnormal in aged human subjects as well as in demented subjects with a diagnosis of Alzheimer's disease.

Our cat model of the human P3 shows a similar polarity, latency, dependence on low stimulus probability or stimulus omission, and is task relevant (Buchwald, J. & Squires, N., In: Conditioning, Plenum Press, N.Y., (C. Woody, ed.) 503-515, 1982). The present study compares the P3 response in 8 young (1-3 yrs) and 13 aged (11-23 yrs) cats. The active electrode was a vertex skull screw, implanted under pentobarbital anesthesia (35 mg/kg), referenced to the neck. Recordings from awake restrained cats were made in a shielded sound isolation chamber. The P3 protocol was a rare: frequent: target schedule of 15%: 80%: 5% in blocks of 500 trials. A conditioning protocol utilized a 4000 Hz tone CS ("target") reinforced with brief shock to the eyelid. This provided a means of focusing attention to the auditory modality without the occurrence of eyeblink conditioned response potentials on the "rare" stimulus trials. Clicks, 15 and 30 dB above auditory brainstem response threshold, were used as rare and frequent stimuli and were randomly presented with probabilities reversed on alternate 500-trial blocks.

All young cats showed a P3 response, defined as a positivity in the 200-500 msec range which was significantly larger to the rare than to the frequent stimulus. In the old cats, by contrast, this response was absent or abnormally small and late.

A principal component analysis (PCA) was carried out on the evoked potentials which occurred 40 to 1000 msec after the rare or frequent loud clicks. Factor scores for each of 6 factors were compared between blocks in which the loud click was frequent versus rare. Three factors showed significant differences. The peaks of these factors had latencies at 40, 60 and 280 msec, which correlated with evoked potential peaks at these same latencies. Evoked potentials from the old cats are currently undergoing principal component analysis.

The data indicate that young cats have a PCA factor at the latency of the P3 potential which shows significantly different factor scores for the rare and frequent stimuli. In the aged cat, as in the aged human, the P3 response shows a marked increase in latency and decrease in amplitude which is being quantified by PCA.
(Supported by USPHS HD04612, HD05958, AG01754 and AG04088.)

347.13 EEG CHANGES IN THE MODERATELY HYPOGLYCEMIC RAT. J. French and D.G. Rawlinson *. Laboratory of Cerebral Metabolism, Cornell University Medical College, New York, N.Y. 10021.

Many investigators have described EEG features characteristic of moderate hypoglycemia while others find no relationship between plasma glucose, EEG and level of awareness. Specific regions of the cortex, striatum and hippocampus have been shown to be selectively vulnerable to hypoglycemic neuronal damage. The present study examines cortical, hippocampal and striatal EEG abnormalities and their responsiveness to sound stimulation during moderate hypoglycemia.

Monopolar electrodes were stereotaxically positioned in 10 adult male rats. After 1 week, the rats were fasted 24 hrs. and their tail arteries were cannulated under ether anesthesia. After 3 hrs. recovery from ether, insulin (Iletin U-40; 4.0-5.0 units/kg) was injected intra-arterially. Arterial glucose was sampled at 15 min intervals throughout 2.0 hrs. of hypoglycemia. EEG records were obtained continouously and behavioral observations were made after each blood glucose sample. Plasma glucose declined rapidly from a baseline mean of 8.7mM to a range of between 3.4 to 1.8 mM within the first hour and gradually between 2.2 to 1.1 mM during the second hour. Mean systemic blood pressure, pH, pO_2, pCO_2 and body temperature were not different from baseline.

It was possible to delineate 2 major stages of moderate hypoglycemia based on clinical arousal and EEG: lethargy and stupor. Lethargy occurred at blood glucose levels between 3.0 and 1.3 mM. The affected animals exhibited intact but slowed clinical orientation to sound. The EEG was characterized by a significant decrease in the incidence of normal brain rhythmic activity ($p < .05$) and by the appearance of large, irregular activity (LIA), occurring at an amplitude of 2 to 4 times the baseline EEG and a frequency of 6 Hz. If the rat was stimulated by sound, the LIA was interrupted and replaced by normal low voltage rhythmic activity. Stupor occurred at blood glucose levels between 1.5 and 1.1 mM. Stuporous rats clinically did not respond to stimulation. The EEG was dominated by a slower frequency of LIA averaging 2 Hz. This pattern was not affected by auditory stimulation. The three brain regions did not differ with regard to the onset and duration of the EEG changes.

These findings indicate that LIA during lethargy can be interrupted by auditory stimulation of the animal. This reactivity of LIA during lethargy but not during stupor further delimits specific EEG changes that occur during moderate hypoglycemia.

347.14 A METHOD FOR OBTAINING AUDITORY BRAINSTEM EVOKED RESPONSES IN UNANESTHETIZED MICE. D.W. Shucard, T.M. Welch*, M.W. Church, S.A. Hoffman. Brain Sciences Laboratories, Department of Pediatrics, NJH/NAC, Denver, CO 80206

The auditory brainstem evoked response (BSER) has become an important clinical tool for assessing the integrity of the auditory pathway in humans. Animal investigations offer the opportunity to study the BSER under well controlled conditions. Of particular significance is the state of the subject (human or animal) being studied. Human BSER recordings typically are made while subjects or patients are either awake or in natural sleep. Animal BSER recordings are most often done with animals sedated or anesthetized. Although anesthesia affords control over artifacts due to movement or muscle activity, the use of anesthesia also leads to difficulties such as hypothermia. Hypothermia readily occurs in small animals such as the laboratory mouse and has been shown to affect the BSER. It also has been shown that anesthesia may affect both the latency and amplitude of the BSER.

We have developed a technique for obtaining BSERs in unanesthetized mice. Stainless steel screws are implanted in the animal's skull and connected via insulated wires to a plug which is permanantly affixed to the animal's head. A threaded female brass post is also affixed to the animal's skull at the midline slightly posterior to the lambda. After recovery and prior to recording the BSER, the mouse is placed in a plexiglass restraining device. This device completely immobilizes the animal's head and maintains the body in a fixed position via: (1) a male screw that is threaded into the post previously affixed to the animal's skull, (2) an adjustable padded collar that lends additional support to the animal's head, and (3) a cylindrical plexiglass structure that encompasses the animal's body. The BSER is then recorded via the head plug. Using this technique we have obtained reliable BSERs which are composed of 4-6 components occurring within 8 msec post stimulus. Recordings are obtained rather quickly, since the animals remain quiet in the restraining apparatus. This procedure allows for the chronic recording of BSERs as well as other electrophysiological responses in the mouse without the complications produced by anesthesia.

347.15 TWO TYPES OF RHYTHMICAL SLOW ACTIVITY (theta) IN THE HIPPOCAMPUS OF NEONATALLY DECORTICATE RATS. Ian Q. Whishaw, Richard Dyck* and Bryan Kolb. Dept. of Psychology, Univ. of Lethbridge, Lethbridge, Alberta T1K 3M4.

Long-Evans hooded rats were decorticate within a few hours of birth. When adult, they were implanted with recording electrodes in the hippocampal formation. Slow-wave recordings were made during behavioral immobility, head movements, turning, rearing and walking, quiet sleep and rapid eye movement (REM) sleep. The rats were also anesthetized with ether or drugged with atropine sulfate (50 mg/kg). The relations between hippoccampal EEG activity and behavior in the neonatally decorticate rats were the same as those described for normal adult rats. Large amplitude slow activity (LIA) was present during behavioral immobility and quiet sleep. RSA was present during head movements, turning, rearing, walking, and REM sleep. The highest frequency and amplitude RSA occurred during walking and the eye movement and twitch periods of REM sleep.

Under ether anesthesia slow (4-6 Hz) RSA was recorded. Atropine abolished this activity. Under atropine, RSA was present with head movements, turning, rearing, and walking.

The results show that in the absence of the neocortex, hippocampal EEG has all of the amplitude, frequency, and waveform features that can be observed in normal rats. The results demonstrate that although the neocortex and cingulate cortex may send direct and indirect projections to the hippocampus proper, input from these pathways are not necessary for normal hippocampal EEG activity.

347.16 EVENT-RELATED SLOW POTENTIALS IN URETHANE ANESTHETIZED RATS. J.H. Pirch, M.J. Corbus* and G.R. Rigdon*. Dept. of Pharmacology, Texas Tech Univ. Health Sci. Ctr., Lubbock, TX 79430.

Event-related slow potentials are generated in the frontal cortex of unanesthetized rats in response to stimuli preceding rewarding stimulation of the medial forebrain bundle (MFB; Pirch et al., Brain Res. Bull. 7: 399, 1981). Cortical slow potential (SP) responses to tone stimuli preceding tail shock were recorded in rats anesthetized with urethane (Ebenezer and Thompson, Br. J. Pharmac., In Press, 1983). The study of event-related slow potential mechanisms could be facilitated if conditioning-related SPs do develop in urethane anesthetized animals. In an initial study, rats (n=4) were implanted with Ag-AgCl electrodes for recording cortical SP responses and monopolar electrodes for stimulating the MFB. Optimum stimulation parameters for the SP conditioning study were determined for each rat during self-stimulation sessions. These animals were then subjected to extensive SP training in the unanesthetized state. In trials presented at variable intervals, a 2-sec tone preceded a single 0.5 sec train of MFB stimulation. As previously reported, negative SP responses developed with pairing of the tone and MFB stimulation. The SP responses were of similar wave form and amplitude under urethane anesthesia (1.2 - 1.5 g/kg, i.p.). Other animals (n>20) were implanted with MFB stimulating electrodes; a small bolt was embedded in the implant cap for fixing the head to a stereotaxic frame. Seven to 10 days after surgery, stimulation parameters were determined as above but the animals were not subjected to the conditioning procedure. For SP recording the rats were anesthetized with urethane, a small incision was made to expose the skull over frontal cortex and holes were drilled to allow placement of Ag-AgCl electrodes on the dura. The recording reference was placed subcutaneously. Using either tone or light stimuli and MFB stimulation as reinforcement, the rats were subjected to pseudoconditioning, conditioning, extinction and retraining trials while recording the SP responses. Negative SP responses to the tone or light were minimal or non-existent during pseudoconditioning, developed gradually with pairing, diminished markedly during extinction and returned to maximum amplitude with retraining. In general, these SPs were smaller than those observed in unanesthetized rats. Event-related slow potentials can be recorded from the frontal cortex of urethane anesthetized rats. (Supported by MH29653)

347.17 ANATOMICAL AND PHYSIOLOGICAL CHARACTERIZATION OF THE CORTICAL AREAS IN CTENOSAURA PECTINATA. M. T. González-Estrada*, R. Budelli*, M. Rodríguez-Budelli, M. Briones* (SPON: W. J. Freeman). Unidad de Investigaciones Cerebrales, Instituto Nacional de Neurología y Neurocirugía and Instituto Mexicano de Psiquiatría México, D. F., México.

The characterization of cortical areas in reptiles is important for 2 reasons. First, because the superficial cortices in reptiles correspond to the primitive deep cortical structures in mammals, such as the hippocampus. Second, they lack of circonvolutions and therefore, the interpretation of field potentials is relatively simple. We initiated this characterization in iguanas.

To study the histomorphology of the cortex, we performed Nissl and silver impregnation techniques.

For the physiological studies we designed a head fixation system. Surgery was carried on in temperature lowered iguanas with local anaesthesia and pretreated with phenothiazine.

Electrocorticograms from the dorsal cortex displayed more synchronized and higher amplitude activity than those from the lateral cortex. Correspondingly, the dorsal cortex showed a higher density of somas and an intrincate cytoarchitecture.

Evoked potentials to single supramaximal shocks (0.1 to 1 Hz frequency, 0.05 to 0.1 msec pulse width, and 10 volts intensity) applied to the olfactory tract and recorded from the dorsal and lateral cortices showed shorter latencies in anterior areas and higher amplitudes in intermediate regions in the anterior-posterior axis.

Repetitive stimulation of the olfactory tract provoked afterdischarges of up to 2 min durations. Eventually, after several stimulation periods, spontaneous spikes occurred.

In conclusion, iguanas constitute a suitable model for the study of electrophysiological synchronized activity. Furthermore, the existance of paroxystic type activity encourages us to persue the investigation of this phenomena in order to elucidate basic mechanisms involved in epilepsy.

347.18 VISUAL EVENT RELATED POTENTIALS IN A STROOP TARGET IDENTIFICATION TASK. A.T. Summerfelt, A.M.I. Wagman and K. Bolla-Wilson* Maryland Psychiatric Research Center, Baltimore, MD 21228

Visual event related potential (ERP) changes associated with interference on Stroop color-word reaction time tasks have been reported to occur for N1 (Warren & Marsh, 1979) and P2 (Johnston & Venables, 1982) but not for P300 (Duncan-Johnson & Kopell, 1981). The present study sought to extend previous findings through the use of principal components analysis (PCA) to separate overlapping late components obtained during a Stroop target detection task.

12 normal volunteers participated in this study. Three types of stimuli were used: target congruent (color words printed in the same ink color as the word, 20%), target incongruent (color words printed in a different color from that named, 20%) and background neutrals (noncolor words printed in various colors, 60%). Subjects were instructed to fixate on an LED which was lighted 250 msec prior to the stimulus and remained on for the duration of the recording epoch, read each word and respond by touching a switch after the LED had extinguished if the word was the name of a color. EEG recordings were obtained from FZ,CZ,PZ, OZ,C3 and C4.

Late components corresponding to P300 and CNV were obtained from a covariance PCA with varimax rotation computed over the averaged ERPs for each subject for each condition at each midline electrode site. Similar components emerged from a separate PCA performed on left and right central ERPs. Repeated measures ANOVA with Greenhouse-Geiser correction was used to assess interference and hemispheric effects for each component.

A significant condition effect was revealed for midline P300 (neutral<congruent and incongruent) which was not associated with interference since target identification resulted in similar amplitude enhancement for both color word classes.

An interference effect was obtained for midline CNV. The significant placement by condition interaction indicated that frontal CNV amplitude was larger for incongruent than for congruent stimuli.

No significant hemispheric asymmetry was present for either component.

347.19 COGNITIVE BEHAVIOR PREDICTS LATE EP COMPONENT AMPLITUDE. A.M.I. Wagman, A.T. Summerfelt, K. Bolla-Wilson, W. Wagman & W.T. Carpenter, Jr. Maryland Psychiatric Research Center, Baltimore, MD 21228

Preliminary data (Soc. Neurosci. Abstr., 1980) obtained upon a small sample of normals and schizophrenics suggested that baseline to peak measures of P300 amplitude and latency were related to measures from the cognitive battery. The present study is an attempt to elucidate these relationships with a larger sample and a principal component (PC) approach on the EP data. 30 schizophrenic and 30 normal subjects were tested on the same day on a cognitive battery and in a visual EP target detection task in order to determine whether cognitive performance is predictive of EP. The cognitive battery consisted of the Wisconsin Card Sort (WCS), Halstead Category Test (HCT), Full-Range Picture Vocabulary (PVT), Pegboard (PEG), Tapping (TAP), Tactual Performance (TPT) and Memory for Designs (MFD). The EP procedure randomly presented targets (20%), backgrounds (60%) and novel (20%) stimuli. Recordings were made from bilateral frontal, central & occipital sites. The EP covariance PC analysis with varimax rotation isolated 5 factors: slow wave (SW), P338, P466, N146/P178, and N114. The cognitive PC using the correlation matrix with varimax rotation yielded 7 factors: C1-HCT, TPTmem, PVT; C2-Peg; C3-TPT & MFD; C4-HCT & WCS unique errors; C5-Tap; C6-TPT, WCS correct & trials; C7-WCS interruption & perseveration errors. Between group differences were found for P338, C1, C2 and C3. These differences indicate that normals produce higher P338 factor amplitude and better C1, C2 and C3 scores.

In order to best characterize the relationship of the EP factors to the cognitive predictors, best subset regression methods minimizing Mallow's Cp criterion were used. The EP factor scores which served as outcome measures were those which previous analyses had demonstrated best described the activity of the factors across conditions and placements.

14% of the frontal target SW variance is accounted for by an equation including C1 and C3. This relationship indicates that good concept identification is associated with SW activity. There was no relationship between cognitive performance and novel SW or P466. 24% of the variance of central P338 was associated with an equation including C2, C3, C5 and C6. These data replicate the preliminary findings of P300 amplitude relationship to MFD and extend the association to other visuo-motor integration measures.

347.20 SURFACE TREND ANALYSIS OF MULTI-CHANNEL EEG IN NORMAL SUBJECTS AND PSYCHIATRIC PATIENTS. J. Metz, P. Tueting, and H. Y. Meltzer. Department of Psychiatry, University of Chicago, Chicago, IL 60637

Multi-channel computerized collection of EEG data has become widely available. Convenient display techniques have also been developed. In an effort to quantify the patterns of activity in different electrodes which can be found in the kind of data, we have applied the geographical technique of surface trend analysis. EEGs were derived from a group of 14 normal subjects and 21 psychiatric patients (6 schizophrenic, 7 depressed, 4 manic, and 4 other diagnoses by RDC).

EEG data was collected from 16 channels by a PDP 11/23 laboratory computer. The power spectrum at each electrode location was determined off-line using the Fast Fourier Transform. Color-codings of values within designated frequency bands at each electrode location and interpolations between electrodes were projected onto a two-dimensional representation of the head. Regional distribution of power was then analyzed by a least squares fitting of the log of the power as a function of the projected x- and y-coordinates, according to the linear equation: $P(i)=A_0+A_1X(i)+A_2Y(i)+R(i)$, where X, Y, and P are the coordinates and log transformed power at each electrode location; A_0 is the intercept at the origin (arbitrarily set at the left posterior corner of the head); A_1 is the rate of change in power in the left-right direction; A_2 is the rate of change in the back-front direction; and R is the difference between the calculated and the observed power at each electrode (residual). In addition to the three coefficients, we determined the percentage of the total variance which could be attributed to the best fitting linear equation and which electrodes showed the largest residuals from that equation in each subject.

Subjects were tested while resting with eyes closed. We examined power in the 8-12 hz bandwidth (alpha) because of reports in the literature that this range is abnormal in psychiatric patients. There were no statistically significant differences between the patients as a whole and the normal subjects. However, there was a clear tendency for the manic subjects to show less frontal spread (high A_2) and better overall linear fit than the normals--the other patient groups were intermediate on both of these values but with high inter-subject variability.

We had expected these analyses to relate to hypothesized hemispheric differences in patient groups (differences in A_1). That expectation was not confirmed. A resting EEG, however, is probably not the best condition in which to look for such differences. The analytic technique should be applied to subjects engaged in different types of tasks.

Supported by USPH MH18396, MH30939, and Ill. Dept. Mental Health.

347.21 ENHANCED SEMANTIC PROCESSING WITH NEGATIVE SLOW POTENTIALS FROM PARIETAL CORTEX. S. C. Whipple* and J. S. Stamm. Department of Psychology, SUNY at Stony Brook, Stony Brook, NY, 11794.

Subjects (university students) were tested on a choice reaction time (RT) task with visually presented word pairs that were either synonyms or unrelated in meaning. EEGs were recorded from midline frontal (Fz), central (Cz), and parietal (Pz) electrodes with a 31-sec time constant. One EEG channel (either Fz or Pz) was monitored for the occurrence of a slow potential (SP) shift. Before each trial, a computer calculated a 2.5 sec average baseline voltage and then generated a 5 sec negative or positive going reference voltage. When the subject's digitized EEG met this voltage criterion, the stimulus word pair was presented. Three groups (8 subjects in each) were tested with SP shifts from Pz or Fz electrodes or as yoked controls.

Intrasubject comparisons showed significantly faster RTs by the Pz group with negative than positive SP shifts, while no RT differences were obtained with the Fz group or yoked controls. Also, the standard deviations of the RTs were significantly smaller with negative SP shifts for both Pz and Fz subjects. These findings comport well with those of a previous study (Born et al. EEG clin Neurophysiol, 54: 668, 1982) where RTs to a geometric configuration task resulted in faster responses with negative SP shifts from the Fz, but not from the Pz electrode.

The findings are evaluated in relation to the various stages of choice RT performance. According to this interpretation, the Pz enhancement reflects faster processing during stimulus categorization in parietal cortex, while the previous Fz enhancements indicate more efficient processing during response selection and programming. The latter effect is also indicated in the present experiment by the reduced SD of the RTs.

347.22 THE EFFECTS OF MOVEMENT AND BODY TENSION ON VISUAL EVOKED POTENTIALS IN NORMAL HUMAN SUBJECTS. R. E. Steenhuis* and L. S. Leung. (SPON: C. H. Vanderwolf). Dept. of Psychology, University of Western Ontario, London, Canada N6A 5C2.

The effects of movement during the acquisition of visual evoked potentials (VEPs) in human subjects have not been extensively examined. The primary effect of movement on auditory and somatosensory evoked responses in both human and animal studies has been suppression of the late waves.

In the present experiment 3 males and 5 females ranging in age from 18-21 years served as subjects. Using a Grass photic flash stimulator, visual evoked potentials were recorded from the occipital lobe (O1) with a forehead reference during both no-movement and several movement conditions (see below). The data was stored on tape and later averaged by a microcomputer.

An increase in amplitude was found in the positive peak at a mean latency of 200 msec (P200) with movement and tension. Other components of the VEP (e.g. N140 and N340) were not consistently affected by movement. Finger movement, either left or right side toe movement and general body tension were effective in enhancing the P200 wave while tongue movement had a variable effect and thinking about finger movement caused a slight suppression. No effects on peak latencies were found for any components of the VEP.

The results indicate a previously uninvestigated interrelation between visual evoked potentials and movement. This specific relation between evoked potentials and movement may be valuable in the diagnosis of movement disorders.

(Supported by NSERC grant U0052.)

347.23 SIMULTANEOUS PET SCAN AND EEG TOPOGRAPHY J.C. Wu,* M.S. Buchsbaum,* H.H. Holcomb, L.E. Delisi,* E. Hazlett,* R. Ball* Department of Psychiatry, University of California, Irvine; Lab. Psych., NIMH (SPON: W.E. Bunney)

Local cerebral glucose uptake with 18_F Fluoro-deoxyglucose (FDG) was assessed by PET with simultaneous topographic EEG which was quantitated by spectral analysis in unmedicated schizophrenics and age and sex matched normal controls. Eight patients with schizophrenia were diagnosed and maintained off medications for a minimum of 14 days (average 39.8 days) by the clinical units of NIMH (Drs. Post, Cohen, Morihisa, Weinberger, Pickar) and the MD State Research Institute (Dr. Carpenter).

Six normals and eight patients received 3-5mCi of FDG while resting with eyes closed in a darkened and acoustically attenuated room. Simultaneously EEG was recorded from 16 channels on the left hemisphere including all 10-20 sites, midline Fz, Pz, Cz and Oz and 4 additional posterior locations for the first 20-30 minutes after injection. Power spectrum of the EEG were computed by fast Fourier transform. Subjectes were then transferred to the Ortec Ecat II scanners and six to seven scans parallel to the CM (canthomeatal) line were made. Using digital techniques; a 2.2 cm thick cortical strip was peeled off and placed on a lateral cortical equal area projection of the brain with the height determined by the proportion of the height from the CM line to the top of the skull. A topographic EEG lateral brain map was also reconstructed by interpolating the values of the EEG power between EEG leads. Thus, two simultaneously attained lateral brain views, one representing metabolic activity and the other representing electrophysiologic activity was created. The calculated glucose value under each EEG lead was correlated with EEG power for the delta, theta, alpha and beta frequency levels.

In normals, a significant negative correlation between alpha and glucose use was observed and was strongest at the occiput as might be expected. Correlations with other frequencies were not significant. As PET procedures are extremely costly, EEG topography may supplement or substitute in some cases. Taken together, the relationship between these two brain imaging techniques awaits further exploration.

347.24 ENDOGENOUS LIMBIC POTENTIALS IN HUMANS: COMPONENT AND SITE SPECIFICITY. J. M. Stapleton, B. E. Derrick*, P. H. Crandall and E. Halgren. Lab. for Cognitive Neurophysiology, Brain Research Institute, UCLA, and V.A. Southwest Regional Epilepsy Center, Los Angeles, CA.

Large polarity-reversing field-potentials are generated in the human hippocampus, parahippocampal gyrus, and amygdala during situations which also evoke the scalp N2-P3-SW (Science 210: 803, 1980). In this study, we recorded from depth electrodes in the human limbic system during tasks which have been found by others to differentially evoke N2, P3 or SW at the scalp. Tasks included: (1) detection of rare targets in a tone series (1400 msec ISI); (2) the same stimuli, but with the patient ignoring the tones; (3) as in task 1, but with occasional strange, task-irrelevant sounds; (4) detection of omissions from a tone series (600 msec ISI); and (5) counting all tones (random 3 to 9 sec ISI). A complete data set was obtained from 10 patients who had electrodes implanted to localize the onset of seizures for surgical treatment. In each patient, field-potentials (0.1 to 100 Hz) were recorded from Fpz, calvarial electrodes in the vicinity of Fz and Cz, and from 19 to 114 depth contacts, all referred to the nose tip.

On the basis of latency, anatomical distribution, and task correlates, it was possible to distinguish 5 depth components roughly correlated with the surface N1-P2-N2-P3-SW. Like the scalp N1-P2, the correlated limbic components occur to both attended and ignored tones, but not to tone omissions. Their polarity is opposite to the surface N1-P2. Overlapping the P2-related component is a large negative limbic potential which, like the scalp N2, is largest to rare attended tones, and has a peak latency of about 240 msec. This limbic component is clearly separable by latency and depth distribution from a component peaking at 350 to 420 msec, usually slightly later than the surface P3. This later component is the only one which clearly reverses polarity across limbic sites. It is usually largest and negative in the anterior or middle hippocampus, and positive in the amygdala or parahippocampal gyrus, although exceptions may be noted. The endogenous limbic components are evoked by tone omissions with onset less than 100 msec after when the tone would have occurred.

These large, polarity-reversing potentials were somewhat specific for both site and task. They were not observed in recordings from orbitofrontal, supplementary motor, or cingulate cortices (1 to 5 patients) or in limbic sites during Readiness Potential or CNV type paradigms. Identification of depth potentials related to the scalp P3 allows us to better relate human cognition and pathology to specific brain activity.

Supported by NS17841, NS02332 and by the Veterans Admin.

347.25 EFFECTS OF INSPIRATORY LOADING ON AUDITORY BRAINSTEM RESPONSES IN MAN. J. Salamy*, M. Grunstein*, D. Shucard, and P. Welanko*. (SPON: M. Church). Brain Sciences Laboratories, Department of Pediatrics, NJH/NAC, Denver, CO 80206

It is generally recognized that in cats and rabbits neuronal activity that covaries with respiration is present from the caudal medulla to the rostral pons. Thus, to a considerable extent, respiratory neurons reside at brainstem levels through which the primary auditory pathway ascends. While the central respiratory mechanisms are not readily accessible in the intact human, the integrity of the auditory pathway can be determined by electrophysiological responses to rapid, brief clicks.

The present study examined whether or not presumed alterations in respiratory neuronal activity induced by inspiratory loading would be reflected as pertubations in the auditory brainstem response (ABR). The ABR was recorded as normal volunteers reclined in a semi darkened sound attenuated room. Standard ABR recording procedures were followed. Respiration was monitored via strain guage placed over the abdomen. Binaural clicks (100 μsec; 65 dB HL; 16/sec) were delivered through headphones as volunteers breathed through a Rudolph valve.

Two groups of 10 volunteers were given 4 trials each of approximately 6 minutes duration. Successive trials were approximately one minute apart. During each trial ABRs were averaged to 2048 clicks which were acquired only during the inspiratory phase of breathing. A control group received no resistive loads on any of the 4 trials. An experimental group was given qualitatively discernable inspiratory resistances of increasing difficulty on the 2nd and 3rd trials, with the 4th trial returning to the original no load condition.

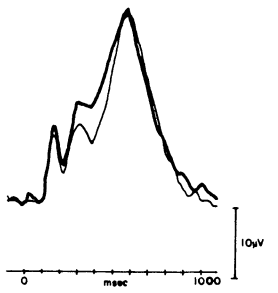
Between group differences in the latencies of the first six peaks of the ABR did not differ statistically across trials. This finding indicates that loading did not interfere with auditory transmission time. Inspiratory loading, however, produced potentiation of the amplitude of the P5-N5 component. Expressed as per cent change from baseline (trial 1), the experimental group produced average amplitude increments of 23%, 19%, and 24% for successive trails. In contrast, the control group showed progressive average reductions in P5-N5 amplitude of about 3%, 9%, and 15% for successive trails. These between group differences were statistically significant (analysis of variance, $p < .02$).

It is presently not clear whether the ABR amplitude potentiation is a direct result of altered respiratory neuronal activity. It is clear, however, that this phenomenon is not due to non-neural events, such as changes in middle ear pressure associated with respiratory load. Further studies are needed to determine the etiology of this respiratory - ABR relationship. The ABR, as used in the manner described here, may prove useful in the study of respiratory neurophysiology in man.

347.26 HUMAN EVENT-RELATED POTENTIALS ASSOCIATED WITH SEMANTIC PRIMING. S. Bentin*, G. McCarthy* and C.C. Wood. (SPON: T. Allison) VA Medical Center, West Haven, CT 06516 and Yale U., New Haven, CT 06520.

When asked to decide if a character string is a word or non-word, subjects respond more quickly to words which were preceded by semantically related words than by unrelated words or nonwords. This priming effect is thought to reflect an excitatory effect exerted by a word within an associative net of related words. As a first step in investigating the neural systems involved in these semantic processes, we studied event-related potentials (ERPs) measured at the scalp in subjects engaged in a priming task. Sixteen subjects were presented with a randomly intermixed list of words and nonwords. Half were nonwords with legal English construction, the remaining half comprised three stimulus categories. Primes and targets were semantically related pairs with the primes immediately preceding the targets in the list. The primes and targets were matched for word frequency, and were reversed in order for half of the subjects so that across subjects each member of the pair served as both prime and target. Fillers were words unrelated to any other words in the list. One word was presented every 2500 msec with the subject required to indicate via button press whether a word or nonword occurred. ERPs were recorded from 16 scalp locations and digitized at 250 Hz for an epoch of 1600 msec starting 100 msec prior to stimulus onset and averaged according to the four stimulus categories. Faster reaction times to targets than primes verified that priming occurred.

The ERPs elicited by targets differed significantly from primes and fillers, which did not differ from each other. Grand mean ERPs from a midline posterior scalp electrode (Pz) for the target (thick) and prime (thin) categories are presented in the figure. The ERPs diverge at about 250 msec and reconverge at about 600 msec following stimulus presentation with the targets more positive than the primes. This difference was largest over the centro-parietal scalp.



347.27 SUBCORTICAL NEGATIVITY ASSOCIATED WITH ENDOGENOUS P300 IN MAN. C. D. Yingling and Y. Hosobuchi* Langley Porter Psychiatric Institute and Dept. of Neurosurgery, University of California, San Francisco, CA 94143.

The P300 component of the human event-related potential (ERP) is an endogenous positive wave with a latency of 300 msec or more which is typically elicited by rare target stimuli in a detection task. Since P300 is clearly related to cognitive rather than to sensory processes, it is of great theoretical significance to determine the neural structure(s) responsible for its generation. Halgren et al (Science 210:803, 1980) recorded a polarity reversal between the hippocampus and parahippocampal gyrus, and have proposed that P300 is generated in the hippocampus. However, Wood et al (Soc. Neurosci. Abstr. 8:976, 1982) reported that unilateral mid-temporal ablations produced no asymmetry of P300, in contradiction to the hippocampal hypothesis.

We have recorded endogenous activity from electrodes in the thalamus and periaqueductal gray (PAG) of a chronic pain patient during auditory and visual P300 tasks, and here report a negative endogenous component from these sites which covaries in latency with the scalp P300, suggesting a more medial, possibly thalamic, generator for P300. Multicontact platinum electrodes were implanted in the right somatosensory thalamus (VPL) and bilaterally in the PAG. We recorded ERPs during an evaluation period before the electrodes were permanently internalized. The location of the electrodes was confirmed both radiologically and by the presence of a short-latency somatosensory response localized to one contact of the VPL electrode and elicited only by contralateral finger stimulation.

Auditory and visual "oddball" tasks were employed; the subject detected rare (20%) tones of lower pitch or dimmer flashes either by pressing a button or silent counting. Responses to auditory stimuli show a clear scalp P300 only to the rare tones, and a negative wave at all subcortical sites with the same latency and target dependency, whether or not an overt response was required. Visual responses show a scalp P300 to rare flashes which had a latency of 450, rather than 300 msec; again, a subcortical wave with the same latency was seen only in target responses, which also was negative at all leads. These data are inconsistent with the hypothesis of a hippocampal origin for P300, since all the subcortical sites were above the level of the hippocampus and would have recorded a positivity if the generator were there. The findings are consistent with a more dorsal and medial origin, possibly in structures of the reticulo-thalamo-cortical arousal system as proposed by Desmedt (EEG Journal 47:648, 1979).

347.28 MACROPOTENTIALS RECORDED FROM THE CINGULATE CORTEX AND ANTERIOR THALAMUS IN RABBITS DURING THE "ODDBALL" PARADIGM USED TO ELICIT P300 IN HUMANS. M. Gabriel, S. P. Sparenborg*, and E. Donchin . Dept. of Psychology, Univ. of IL, Champaign, IL 61820.

The increasing use of the P300 and other components of the human event-related potential (ERP) as tools in the study of human cognition has stimulated interest in the development of animal models for study of the neural origins of these potentials. Here, we report initial studies in which responses that may be analogous to the human P300 were recorded from rabbits. It has been demonstrated by Gabriel and his associates that neurons in the cingulate cortex (area 29) and the anterior ventral (AV) thalamic nucleus in rabbits develop larger discharges to a CS+ (a tone predictive of footshock) than to a nonpredictive stimulus (CS-), during the course of discriminative avoidance conditioning. This discriminative activity suggested that rabbits may be sensitive to the variables that control P300 amplitude. In the present study, rabbits received daily training sessions (120 trials, 60 CS+ and 60 CS-, in a random sequence) to a criterion requiring performance of the conditioned response (locomotion in an activity wheel) on >70% of the CS+ trials and on <10% of the CS- trials (mean interval between CS presentations (ISI) = 16 sec; mean behavioral latency = 3.2 sec). After completion of the criterial session, an additional "oddball" session was given in which the CSs were presented with a brief ISI (4-6 sec). The CS+ in this session occurred on only 20%, and the CS- on 80% of the trials. The few behavioral responses that occurred at the outset of the session halted the procedure. No footshocks were delivered during the oddball session. On the next day, an additional standard training session was given, followed by an oddball session. This time the CS+ and CS- occurred 80% and 20% of the time, respectively. During the standard sessions, ERPs from probes in the cingulate cortices and AV nuclei manifested a positive wave with a peak latency ranging from 150-300 ms in response to CS+ and CS-. Several records showed later negative and positive waves encompassing the full 700 ms analysis period. During the oddball session in which the CS+ served as the rare event, the waves elicited by the frequent stimulus (CS-) were considerably smaller than those elicited by the rare stimulus and they were smaller than the waves in the standard sessions. When the CS+ was frequent, both stimuli elicited waves equal in amplitude to those obtained in the standard session. There appears thus in the rabbit a pattern of responses analogous in a number of important respects to the pattern labeled P300 in the human ERP. The details of the analogy are now undergoing examination.

347.29 ANALYSIS OF LATE-LATENCY AVERAGED EVOKED POTENTIALS TO ELECTRICAL STIMULATION OF THE TONGUE IN MAN. R. Leppanen and R. Stiles. Dept. of Physiology, Univ. of TN Ctr. Hlth. Sci., Memphis, Tennessee 38163.

Human long-latency evoked potentials (EPs) have been studied for some time but there are few hypotheses that define their source(s) from within the brain (Galambos and Hillyard, Neurosci. Res. Pgm. Bull. 20:141-265, 1981). These EPs are similar for different stimulus modalities, suggesting a common underlying process. EPs (64 sweeps) were recorded in 16 normal human subjects following electrical stimulation of the tongue. Averages were recorded at CZ, C5 and C6 with inion reference with a 54 msec preanalysis time and a 446 msec post-stimulus window with subjects either attending or ignoring the stimulus. Stimulation was at 10 sec intervals with a mild tingling sensation without pain being reported. Major components present in the CZ EP were: P1, N1, and P2 with mean group latencies of 97, 146, and 223 msec, respectively. In C5 and C6 EPs, similar N1, P1, N2, P2, N3 and P3 components were obtained with mean group latencies of 82, 110, 141, 182, 226, and 296 msec, respectively. A major finding was that the waveform components upon tongue stimulation are very similar to those recorded in humans as a result of electrical tooth pulp stimulation (Chatrian, et al, Neurology 25:745-757, 1975). Spectral analysis showed 2 major bands (2-7 and 10-16 Hz), the power of which was increased with the subject attending. Separation of the components with Inverse Fourier Transform revealed 4 waveforms which are similar to the synchronized-desynchronized EEG, the EP and the slow waves described by Skinner and Yingling (Prog. Clin. Neurophysiol. 1:30-69, 1977) in their model of the regulation of sensory input into the cerebral cortex in animals. It is concluded that: (1) The long-latency EPs can be decomposed into different waveforms in the time domain. (2) The timing and changes in amplitude of the filtered components with changes in attention are consistent with Skinner's model and these waveforms may result from processes similar to those proposed by Skinner and Yingling. (3) The EPs obtained from humans as a result of electrical tooth pulp stimulation do not necessarily indicate processes specific to the sensation of pain.

347.PO EVOKED POTENTIALS AND SPINAL CORD BLOOD FLOWS IN TRANSECTED RATS. J.C. de la Torre, M. Gonzalez-Carvajal, N. Hayashi, Div. of Neurosurgery, Northwestern Univ. Medical School, Chicago, IL 60611

A physiological study using somatosensory evoked potentials (SEP) and local spinal cord blood flow (lSCBF) was done 90 days after total transection of rat spinal cords. Male Long-Evans hooded rats 350-450g were anesthetized then subjected to a 200g/cm force contusion injury on T_{10} dorsal spinal cord. Ten days later, rats were transected at T_{10}. Rats were randomly divided into 3 groups A,B,C. Group A underwent laminectomy only without trauma or transection. Group B had the proximo-distal cord stumps realigned end to end following transection. Group C had 2 mm tissue trimmed from the proximo-distal stumps and had the gap filled with a cell-free, bovine derived collagen matrix. This collagen matrix is dispensed from a syringe at 4°C as a fluid which then hardens into a firm, rubbery gel at 37°C within an hour. The collagen matrix appeared to form a tight, structural continuity with the proximo-distal stump junctions, in effect, creating a loosely fibrous bridge following polymerization. All rats were examined daily and their neurological status was recorded for 90 days. At the end of the observation period, all rats were prepared for bilateral SEP. Control SEP was obtained on each rat by stimulating both median nerves above the lesion, then below the lesion using a posterior branch of the femoral nerve (saphenous) while recording from left and right skull electrodes. Following SEP, Teflon-insulated microelectrodes were stereotaxically placed into the cord tissue and lSCBF was recorded at five sites near the lesion using the hydrogen clearance method. Results show that 5 of 20 rats in Group C and 0 of 12 rats in Group B (control) had several early SEP peaks ranging in latency from 9-32 m sec recorded unilaterally. One of the 8 rats in Group C had similar early SEP peaks recorded bilaterally. Mean lSCBF in Group C rats showed blood flows ranging from 29 (always expressed as ml/100g tissue/min) at the center of the collagen matrix to 40-66 at the collagen:cord tissue junction and 5 mm from the center respectively. Group B had mean lSCBF of 10 at the center of the lesion and 24-61 using similar recording sites to Group C. Group A rats (sham injury) showed a normal 7 peak SEP within a time base frequency of 80 m sec and normal mean lSCBF ranging from 49-68 in sites similar to Groups A,B.

These results indicate that incomplete sensory conduction and presumably adequate blood flow can be obtained across severely injured-spinal transected rats using a metamorphic, non-tissue collagen matrix. In spite of these improvements over controls, no rat in any lesioned Group regained functional walking ability.

348.1 INCREASED REM SLEEP IN RATS AFTER CHRONIC TREATMENT WITH THE ACETYLCHOLINESTERASE INHIBITOR DI-ISOPROPYL-FLUOROPHOSPHATE. James Gnadt and G. Vernon Pegram, Neurosciences Program, University of Alabama in Birmingham, Birmingham, Alabama, 35294.

Cholinergic mechanisms are thought to be involved in the mechanisms which control rapid eye movement (REM) sleep and a model describing this mechanism has been proposed (1). By inhibiting the enzyme which degrades the neurotransmitter acetylcholine, it is possible to potentiate cholinergic activity at cholinergic synapses. Thus, it should be possible to effect REM sleep with an acetylcholinesterase inhibitor (anti-AChE).

To test this prediction we have performed the following experiment. Adult rats (250-350 g) were implanted with electrodes to record neocortical and dorsal hippocampal EEGs. The hippocampal EEG was used to obtain hippocampal theta (7-8 Hz) which distinguishes REM sleep from slow wave sleep (SWS) and awake. Muscle activity was determined by implanting bipolar EMG electrodes in dorsal neck musculature and/or movement was recorded from a free, ungrounded lead attached near the rat's head. After at least 4 days for recovery from surgery, we used the "irreversible" anti-AChE, di-isopropyl-fluorophosphate (DFP), to chronically reduce acetylcholinesterase activity in rats (2). DFP in arachis oil was injected i.m. at 1.0 mg/kg initially and 0.5 mg/kg every third day thereafter for a total of 5 injections (12 days). The second day following the last injection the rats were recorded for 7½ hrs. (0830-1600) to determine the effects of DFP on the sleep-wake cycle. The records were scored visually by 50 sec epochs into awake, SWS, and REM sleep.

Following the injection regimen, DFP-treated rats exhibited several indices of increased REM sleep: increased % REM, increased # REMs, shortened inter-REM-sleep interval. The mean of the percent of REM sleep for the DFP-treated rats (14.3 ± 0.37, $\bar{X}$ ± SEM) was significantly higher than the mean for the vehicle-control rats (12.5 ± 0.69); one-tailed Mann-Whitney U, $p < .025$.

These data are consistent with the view that cholinergic mechanisms are involved in REM sleep. These data also compliment the findings of other investigators who have studied the effects of anti-AChEs on sleep (3,4,5). (Supported by USAMRDC contract DAMD17-83-C-3040.)

1) McCarley, R.W. & Hobson, J.A. Science 189:58-60, 1975.
2) Glow, P.H., et al. Aust. J. Exp. Biol. 44:73-86, 1966.
3) Karczmar, A.G., et al. Physiol. Behav. 5:175-182, 1970.
4) Gillin, J.C., et al. Psychopharmacol. 58:111-114, 1978.
5) Duffy, F.H. & Birchfiel, J.L. Neurotoxicol. 1(3):667-689, 1980.

348.2 PHENOXYBENZAMINE INDUCED REMS SUPPRESSION IN RATS IS TEMPORALLY RELATED TO AN ELEVATION IN MHPG-SO_4, BUT NOT TO A DECREASE IN THE DENSITY OF α_1 BINDING SITES. R. C. Walovitch and M. Radulovacki, NIDA Addiction Research Center, Baltimore, MD. 21224; Dept. of Pharmacology, Univ. of Ill. Medical Center, Chicago, IL. 60612.

The aim of this study was to determine if a temporal relationship exists between long lasting suppression of REM Sleep (REMS) and suppression of α_1 noradrenergic transmission produced by phenoxybenzamine (Pbz) in rats. The following experiments were performed: Rats in one group were implanted with EEG and EMG electrodes and were continuously polygraphically recorded for 60 hr following Pbz (10 mg/kg, i.p.) administration. Rats in the other group were killed, and their brain were removed 12, 24 or 60 hr. after Pbz (10 mg/kg, i.p.) administration. Each brain was sectioned transversely between the inferior and superior colliculi. The posterior portion was discarded and the anterior portion was considered as forebrain. Halves of the forebrains were used for assay of [^{3}H]prazosin binding; tissue from the other hemisphere was used for 3-methoxy-4-hydroxy phenylethylen-glycol sulfate (MHPG-SO_4) (a major central noradrenergic metabolite) determination.

Tissue from Pbz-treated rats showed decreases in the density of [^{3}H]prazosin binding sites and elevation in MHPG-SO_4. These effects may reflect irreversible binding of Pbz to α_1 receptors and a functional decrease in noradrenergic transmission respectively.

After Pbz administration, REMS was suppressed and MHPG-SO_4 was elevated for approximately 48 hr indicating a temporal relationship between these two phenomena. In contrast, the ability of Pbz to decrease the number of available [^{3}H]prazosin binding sites was still apparent 60 hr after Pbz administration (i.e. B_{max} for [^{3}H]prazosin binding in Pbz treated animals was approximately half of control) at a time when both REMS and MHPG-SO_4 had already returned to normal levels. This discrepancy could be explained by the Spare Receptor Theory, which postulates that a maximum response may be elicited with only a small number of receptors occupied.

Supported by ONR Contract N0014-79-C-0420

348.3 EFFECT OF CLONIDINE ON EEG WAVEBANDS ASSOCIATED WITH SLEEP IN THE RAT. Ross H. Pastel* and John D. Fernstrom. Western Psychiatric Institute and Clinic, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

Recently, we have developed a microcomputer-based system for detecting and quantitating in rats the EEG waves most commonly associated with the sleep-waking cycle. This system has been applied to the study of clonidine, which is most often reported to suppress REM sleep and increase REM latency. Administration of clonidine (0.001 - 1.0 mg/kg,ip) at the onset of the daily light period rapidly suppressed cortical delta wave occurrence. Subsequent rebounds occurred, earlier for the smaller doses (0.01 mg/kg dose in table) and later for the larger doses. Clonidine injection also produced an acute, dose-related, biphasic effect on spindles: at low doses it appeared to suppress, while at high doses to enhance spindle occurrence (see table).

Dose	Delta		Spindles	RSA
(mg/kg)	0-4 hr	5-8 hr	0-1 hr	0-1 hr
0.00	100±10	100±14	100±34	100±11
.001	80±19	111±7	98±46	100±11
0.01	44±9	142±19	73±22	128±16
0.10	58±14	89±14	165±26	120±12
1.00	61±14	90±26	171±48	67±10

[Data are % of saline control values (0.00 mg/kg), expressed as means ± sem; n = 6/dose group, except at 0.001 mg/kg (n = 3).]

Clonidine also had an unusual effect on rhythmical slow activity (RSA) in the hippocampus: It induced low frequency (4-7 Hz) RSA at all doses above 0.001 mg/kg. This effect persisted up to 20 hr at the highest dose. RSA counts shown in the table are within a 5-10 Hz waveband. At the 1.0 mg/kg dose, RSA still dominated the EEG record, but there was a decrease in computer counts of RSA. This suggests that at this dose, clonidine also induced a decrease in the frequency of the RSA, to less than 5 Hz.

If these data were analyzed using standard sleep-scoring criteria, both REM and SWS would appear to have been suppressed. However, the behavioral state was not a normal waking state. Clonidine induced an EEG pattern characterized by an unusual combination of cortical spindles and low frequency hippocampal RSA. These results suggest that in rats, effects of clonidine cannot readily be interpreted as an alteration in one or more of the classically-defined sleep states. Further, they raise the question of whether the commonly reported suppression of REM sleep by clonidine is a direct effect of the drug, or is instead the result of the induced behavioral state and the associated disruption of normal slow wave sleep.

[These studies were supported by a grant from the NIMH (MH38178).]

348.4 ADULT PROGENY OF RATS PRENATALLY EXPOSED TO DIAZEPAM HAVE MORE SLOW WAVE SLEEP I AND LESS SLOW WAVE SLEEP II. G.T. Livezey M. Radulovacki, T. Baglajewski* and T.J. Marczynski, Dept. of Pharmacology, University of Illinois College of Medicine, Chicago, IL 60612.

A previous study in cats has shown that prenatal exposure to low doses (max. 0.6 mg/kg) of diazepam results in impairment of a specific form of slow wave EEG known as Post-Reinforcement Synchronization as well as regionally specific reductions in the number of brain benzodiazepine (BZ) receptors (1). Although the role of BZ receptors in sleep is not well understood it has been suggested that total sleep deprivation may enhance BZ binding (2) while neither REM deprivation nor REM rebound has any effect on BZ binding (3). The present experiment examined the effects of prenatal exposure to diazepam on the sleep patterns and BZ binding of the adult rat progeny. Female Sprague Dawley rats were injected once daily (s.c.) with either diazepam or its vehicle according to the following schedule:

GESTATIONAL DAY	1-14	15	16	17	18	19	20	21
DOSE (mg/kg)	none	5.0	5.0	7.5	7.5	5.0	5.0	none

The progeny were reared to 120 days of age, then implanted for EEG and EMG recording, and recorded for 24 hours continuously. The records were scored for wakefulness, slow wave sleep I (SWS_1), slow wave sleep II (SWS_2), and REM sleep.

TABLE I

	W	SWS_1	SWS_2	REM	TOTAL SLEEP
C	613.6 ± 120.4	288.9± 43.9	393.2±69.9	135.3 ± 21.2	826.5 ± 120.4
DZ	568.1 ± 48.1	440.0± 48.8*	282.9±29.1*	149.2 ± 26.0	871.9 ± 48.1

All values are means of 10 animals ± S.D. *SWS_1 $P<.00005$, SWS_2 $P<.001$

These data indicate a highly significant shift of slow wave sleep from the deeper SWS_2 to the lighter SWS_1 without changes in wakefulness, REM sleep, or total sleep in rats prenatally exposed to diazepam. In addition, there was fragmentation of SWS_2 into short bursts in the diazepam group. These changes in sleep could be related to a 24% reduction in BZ receptor number in whole cortex samples of rats in the diazepam group with respect to controls (preliminary data-not shown here). The findings suggest a role for benzodiazepin receptors in the regulation of sleep states. (Supported by ONR Contract N00014-79-C-0420).

References

1) Livezey, G.T., Isaac, L., and Marczynski, T.J.: Neuroscience Abstr. 8: 108, 1982; 2) Mendelson, W.B., Gillin, J.C., and Wyatt, R.J.: Advances in Pharmacology and Therapeutics II, ed. H. Yochida, Y. Hagihara, and S. Ebashi, Oxford and New York: Pergamon Press, pp. 227-331, 1982; 3) Mueser, T., Isaac, L., and Radulovacki, M.: Physiol. Behav. (in press).

348.5 ADENOSINE RECEPTOR BINDING IN WHOLE BRAINS FROM YOUNG AND OLD RATS. R.M. Virus, T. Baglajewski*, and M. Radulovacki. Department of Pharmacology, University of Illinois College of Medicine, Chicago, IL 60612.

Recent experimental evidence suggests that adenosine is a neurotransmitter or neuromodulator in the mammalian CNS (1) and may participate in the regulation of sleep and wakefulness (2,3). Since the total amount of sleep is reduced and distribution of sleep states is altered in aged rats (4), the present experiments examined the binding of (-)[^{3}H] N^6-phenylisopropyladenosine ((-)[^{3}H] PIA) to whole brains from young (12 weeks of age) and old (48 weeks of age) normal male Sprague-Dawley rats.

All animals were housed under a 12 h light (0800-2000 h)/12 h dark (2000-0800 h) cycle with free access to food and water for at least one week prior to sacrifice and tissue dissection. Rats were decapitated and brains were rapidly removed and frozen on dry ice until subsequent binding assays between 1100 and 1300 h. (-)[^{3}H] PIA binding was assessed using a previously published method (5). The results obtained are presented in the following table:

	AGE IN WEEKS 12	AGE IN WEEKS 48	PERCENT CHANGE
B_{max} (fmol/mg protein)	787 ± 221	830 ± 208	+ 5.46
K_D (nM)	61.37 ± 14.99	112.75 ± 27.35	+ 83.72

All values reported are mean ± S.E.M. of duplicate determinations with 4 rats per group. No statistically significant differences between groups were detected using Student's t-test.

These data clearly demonstrate that the total number of (-)[^{3}H] PIA binding sites (B_{max}) was nearly identical in both young and old rats but that the dissociation constant (K_D) for (-)[^{3}H] PIA was nearly 84% greater in old as compared to young rats. Although this difference failed to attain statistical significance in these preliminary experiments (P 0.500), it remains possible that the apparent decreased affinity of adenosine receptors observed in old rats may be involved in the decreased amounts and altered patterns of sleep associated with aging in this species (4). (Supported by ONR Contract N00014-79-C0420).

References: 1) Phillis, J.W. and Wu, P.H., Progr. in Neurobiol. 16: 187, 1981; 2) Virus, R.M., Djuricic-Nedelson, M., Radulovacki, M., and Green, R.D., Neuropharmacology in press, 1983; 3) Radulovacki, M., Miletich, R.S., and Green, R.D., Brain Res. 246: 178, 1982; 4) Rosenberg, R.S., Zepelin, H., and Rechtschaffen, A., J. Gerontol. 34: 525, 1979; 5) Schwabe, U. and Trost, T., Naunyn-Schmiedeberg's Arch. Pharmacol. 313: 179, 1980.

348.6 *HYPNOTIC EFFECTS OF ADENOSINE DERIVATIVES, CHA AND NECA IN RATS.* M. Radulovacki, R.M. Virus, M. Djuricic-Nedelson* and R.D. Green*. Department of Pharmacology, University of Illinois College of Medicine, Chicago, IL 60612

Adenosine may participate in the regulation of sleep, since intraventricular administration of adenosine to fowls, cats and dogs (1,2,3), as well as administration of a metabolically stable adenosine derivative N^6-L-(phenylisopropyl)adenosine to rats (4), produced hypnotic effects. Our interest in the possible hypnogenic role of adenosine was stimulated by findings that behavioral stimulant effects of methylxanthines involve a blockade of central adenosine receptors (5). In addition, general neurophysiologic effects of adenosine were shown to be inhibitory (6) and it is conceivable that stimulation of adenosine receptors may produce sedation or sleep. In the present study we examined in rats hypnotic effects of two metabolically stable adenosine derivatives, cyclohexyladenosine (CHA) and adenosine-5'-N-ethylcarboxamide (NECA).

Sprague-Dawley rats implanted with EEG and EMG electrodes were polygraphically recorded for 6 h following administration of CHA and NECA and hypnotic effects of these compounds consisted of: a) increased slow-wave sleep$_2$ (SWS_2) from 6.6 to 45.7%, in all doses used and b) increased values for rapid-eye-movement- (REM) sleep, amounting to 56.2% for 0.1 µmoles/kg CHA. SWS_1 decreased but values for wakefulness and total sleep were unchanged for 0.03, 0.1 and 0.3 µmoles/kg doses of the drugs. Only 0.9 µmoles/kg dose of CHA increased wakefulness and decreased total sleep, while the same dose of NECA increased total sleep during the 0-3 hr time interval. Both agents reduced REM sleep in 0.9 µmoles/kg dose. The results indicate that the hypnogenic effect of both adenosine derivatives was obtained with certain nanomolar concentrations of the drugs and that it diminished or disappeared, when the drugs' concentration reached micromolar range (0.9 µmoles/kg). It appears therefore, that activation of A_1 rather than A_2 receptors contributed to the hypnotic action of the drugs, since adenosine and adenosine derivatives activate A_1 receptors in nanomolar quantities while activation of A_2 receptors requires micromolar concentration of these compounds. The results also indicate that hypnotic effects of adenosine derivatives in rats differ from hypnotic effects of barbiturates and benzodiazepines, because the latter do not increase REM sleep and because increases in SWS_2 and REM sleep by adenosine derivatives were obtained only at certain dosages and could not be further augmented by a dose increase. Supported by ONR contract N00014-70-0420.

References: 1) Marley E. and Nistico, G. Br. J. Pharmac. 46 (1972) 619, 2) Feldberg, W. and Sherwood, S.L., J. Physiol. 123 (1954) 148, 3) Haulica, I., et al., J. Neurochem. 21 (1973) 1019, 4) Radulovacki, M., et al., Brain Res., 246 (1982) 178, 5) Snyder et al., Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 3260, 6) Phillis et al., Can. J. Physiol. Pharmacol. 57 (1979) 1289.

348.7 SLEEP STATE SPECIFIC NEURONAL ACTIVITY IN RAT DORSAL LATERAL GENICULATE NUCLEUS IS NOT ALTERED BY LOCAL SEROTONIN AND NOREPINEPHRINE DEPLETION. H. P. Roffwarg, S. G. Speciale and G. A. Marks. Dept. of Psychiatry, University of Texas Health Science Center, Dallas, TX 75235.

Relay cells of the dorsal lateral geniculate nucleus (dLGN) are one cell population of the many found throughout the neuraxis that alter their spontaneous discharge rate and pattern with changes in state of arousal. Since bilateral eye enucleation does not affect the sleep-related activity in dLGN, central mechanisms are implicated in its control. A common set of central mechanisms may be responsible for the concurrent shifts in cell activity observed in dLGN and other brain areas. However, the neural systems that initiate and propagate sleep-related commands have not been identified.

Our strategy is to systematically and locally remove identified afferents of dLGN to determine which inputs are controlling the tonic sleep-related activity observed in this nucleus. The present study utilizes a microwire cannula guide tube assembly implanted bilaterally in the dLGN and standard electrodes to define the sleep-wake stages. The multiple unit activity derived from the microwires yields REM:SWS ratios of between 2:1 and 6:1. A solution of the monoamine neurotoxin 5,7-dihydroxytryptamine (0.5 ul; 40 ug/ul) was pressure injected slowly through a cannula inserted into the guide tube. The contralateral side received a control solution. Mean frequencies of the multiple unit activity were determined within sleep-wake stages between ipsi- and contralateral dLGNs and during pre- and post drug administration.

HPLC-EC analyses of microdissected dLGN tissue samples from rats sacrificed five days post drug injection revealed mean serotonin levels 14% and mean norepinephrine levels 17% of the control injected side. Despite drastic reduction in monoamine levels, no difference was observed between the sleep-related activity derived from the drug and control injected dLGNs.

Both serotonergic and noradrenergic systems have been hypothesized to be involved in sleep control mechanisms. These data indicate, however, that the integrity of both these system's input to the dLGN is not necessary for the appearance of sleep-related neuronal activity.

348.8 CENTRAL INHIBITION OF ACETYLCHOLINESTERASE PRODUCES A POTENT ENHANCEMENT OF ELECTROGRAPHIC DESYNCHRONIZED (D) SLEEP SIGNS AND BEHAVIOR. H.A. Baghdoyan* F. Assens* M.L.Rodrigo-Angulo* A.P. Monaco* R.W. McCarley and J.A. Hobson. Laboratory of Neurophysiology, Harvard Medical School, Boston, Ma. 02115

The hypothesis that cholinergic pontine reticular formation (PRF) mechansims underlie the generation of D sleep has received support from studies showing that microinjection of cholinergic agonists into the PRF significantly increases electrographic D sleep signs and behavior. The data reported here demonstrate that blockade of the enzymatic breakdown of acetylcholine (ACh) in the pons following microinjections of neostigmine produces an equally potent enhancement of D sleep.

Two cats were implanted with guide tubes aimed at the rostral PRF and with electrodes for recording the EEG, EOG, EMG and PGO waves. Drugs were injected unilaterally with a 1 microliter Hamilton syringe in a volume of 250 nl sterile saline. Controls consisted of baseline (no injection) and saline injection trials. Polygraphic recordings were obtained for 4 hrs post injection.

Neostigmine (20 ug) enhanced electrographic D sleep signs and behavior in all 8 trials (4 sites, 2 cats). The cats spent an average of 72.0 ± 18.6% of the recording period in D sleep, a seven-fold increase compared with 9.6 ± 5.0% during control (N=13) trials. D sleep latency, defined as the time in min from the onset of the injection to the beginning of the first D sleep episode, was reduced four-fold from the control level of 70.8 ± 40.2 min to 17.6 ± 4.0 min by neostigmine. Furthermore, there was a relationship between the dose of neostigmine and the enhancement of D sleep signs, with the lower doses causing a decrease in D sleep latency but no increase in D sleep percent.

The latency of the neostigmine response could be reduced by administering ACh (5ug) with the neostigmine (ACh/N). At one site D sleep latency was cut in half, from 14.1 min with neostigmine to 6.6 min with ACh/N. At another pontine site, a less dramatic decrease in D sleep latency was observed, from 20.1 min with neostigmine to 15.8 min with ACh/N.

These findings are consistent with the hypothesis that D sleep can be triggered and sustained by the accumulation of endogenously released ACh and that D sleep latency results, in part, from the accumulation of endogenous ACh to levels which are sufficient for generating D sleep.

Supported by grants MH 13923, NRSA MH 14275 (HAB) and 1 F05 TW03088-01 (MLR-A)

348.9 PONTINE SITES FOR CHOLINERGIC PGO WAVES AND ATONIA: LOCALIZATION AND BLOCKADE WITH SCOPOLAMINE. P. Shiromani and D. J. McGinty, VA Medical Center, Sepulveda, CA 91343.

A number of studies have shown that infusion of carbachol into the brainstem produces some or all of the tonic and phasic components of REM sleep. These results lend support to the hypothesis that cholinergic mechanisms within the brainstem generate REM. The purposes of this study were to determine if the effects of carbachol are site-specific and if scopolamine is able to block the effects.

Five cats were implanted with standard sleep recording electrodes and a unilateral stainless steel 23 gauge cannula in the rostral and caudal pons, or the medial vestibular nucleus. A push-pull system (rate 9 ul/min) was used, and either Ringers or the drugs were delivered for 40 minutes using the following paradigm: (A) Ringers, (B) carbachol or scopolamine followed by carbachol. The results are summarized in the table below. Carbachol infusions into the rostral pons produced arousal, agitation and intense rage. In fact, arousal was seen even with Ringers infusion. In more caudal areas, incidence of PGO activity and atonia increased but the EEG desynchronization did not change appreciably. Pre-treatment with scopolamine completely blocked the effect of carbachol but did not induce synchronization. Infusion of carbachol into the medial vestibular nucleus produced intense horizontal eye movements without accompanying PGO activation or muscle atonia.

These results support the hypothesis that structures mediating arousal may be located in the rostral pons, while a discrete system responsible for PGO generation and atonia is confined to the caudal pons, and that activation of this system depends upon muscarinic receptor excitation.

Infusion site	% desynch *	# PGO	% atonia *
P=3,L=1.7, carbachol alone			
Ringers	92.3	0	0
Carbachol	81.3	5.3	9.3
40 min post carb	96.4	0	2.5
80 min post carb	100.0	0	0
P=5,L=1.7, carbachol alone			
Ringers	48.8	16.9	3.1
Carbachol	52.8	40.6	18.0
40 min post carb	62.7	63.0	34.4
80 min post carb	69.6	78.2	42.6
scopolamine followed by carbachol			
Scopolamine	57.2	21.0	2.0
Carbachol	74.0	15.9	0
40 min post carb	68.9	27.7	2.4

* percentage expressed as a function of total time

348.10 SLEEP SPINDLES: DENSITY MEASURES CORRELATE WITH INCIDENCE OF INTRASLEEP AROUSALS. S. Scott Bowersox and William C. Dement. Sleep Research Center, Stanford University School of Medicine, Stanford, CA 94305

Our aim in this study was to determine whether age-related changes in the incidence of transient intrasleep arousals in the cat are correlated with changes in the expression of rhythmic, 12-15 Hz EEG spindle patterns. Recent studies have indicated that cortical spindles reduce the probability of transient EEG activation during sleep (Erhart, et al., Sleep, 4:400-407, 1981). We therefore predicted that aged cats, because they tend to exhibit greater numbers of intrasleep awakenings, would have lower EEG spindle densities than young adult animals.

Five aged (9.3-11.2 yrs) and four young adult (2-4 yrs) cats were prepared surgically with indwelling electrodes for chronic monitoring of state-pattern variables. After recovery, 12-h polygraphic recordings of the cortical EEG, EOG, and EMG were obtained. Records were scored for state according to standard criteria. Transient arousals (TA) were identified as brief periods (6-14 s) of EEG desynchronization during non-REM sleep appearing alone or in association with movement. Three continuous 5-min segments of sensorimotor cortical EEG were scaled then subjected to automatic spindle analysis. Signals were passed through a 12-15 Hz filter-relay-logic device (peak signal detection at 13.5 Hz w/6dB down at 12 Hz and 15 Hz); spindles were scored each time filter output met or exceeded minimum amplitude (25μV) and duration (0.5 s) criteria.

Both the incidence of TA's and spindle densities were significantly higher in the aged cats than in the young adult animals. Moreover, spindle densities predicted the occurrence of intrasleep awakenings ($r=0.53$, $p \leq 0.05$).

	Spindles/min SWS	TA/hr SWS
Aged	17.4 ± 4.6*	13.0 ± 2.9*
Young adults	10.7 ± 2.9	5.5 ± 3.5

* $p \leq 0.02$

These findings may be explained on the basis of currently held theories of spindle function. Since spindles are consistently correlated with the attenuation of somatosensory and motor excitability (Sterman and Bowersox, Sleep, 4:408-422, 1981), their facilitation in old age may reflect compensatory thalamocortical responses to pathologic reductions of arousal threshold.

348.11 MEDIAL RETICULAR UNIT ACTIVITY IN THE CHRONIC MEDULLARY CAT. J. M. Siegel, R. Nienhuis* and K. S. Tomaszewski*. Sepulveda V.A.M.C., Sepulveda, CA 91343 and Dept. of Psychiatry, School of Medicine, University of California, Los Angeles, CA 90024

Medial reticular formation (RF) units show dramatic changes in discharge rate and pattern during the sleep cycle of intact animals. Discharge rate is slow in quiet waking and non-REM sleep and high and irregular in active waking and REM sleep. Two behavioral states have been identified in the chronic medullary cat (Siegel et al., Neuroscience Abstracts, 7: p. 233). The predominant state is a "quiescent state" during which respiration and heart rate are regular and nuchal EMG is low. The second state is an "activated state" during which EMG and respiration rates are increased. This state recurs at approximately 25 minute intervals. We have examined medial RF unit discharge in these transected cats to determine how unit activity covaries with these states. We also sought to determine if RF unit activity might reveal state subdivisions not expressed in EMG and respiratory changes. In particular we were interested in seeing if there were periods of RF unit discharge variability resembling those seen in REM sleep and active waking, or periods of increased unit activity in the absence of EMG increase such as are observed in REM sleep.

The brainstems of 3 cats were transected caudal to the locus coeruleus complex and at or rostral to the 6th nerve nucleus. A total of 50 units were recorded in the nucleus gigantocellularis of the brainstem between days 4 and 28 after transection. Discharge rates averaged 14.2/sec during the quiescent state. This rate was significantly greater than that seen in RF units during non-REM sleep in intact cats. This was due to a greater number of cells with discharge rates <1 sec in the intact cats. Rates in the quiescent state were significantly lower than rates in REM sleep in the intact cat. Discharge rates increased an average of 144% during phasic arousals. Unit discharge rates and EMG levels during phasic arousals were comparable to those seen in the intact cat during active waking. Autocorrelation and interval histograms analyses demonstrated that unit discharge rates during the quiescent state were extremely regular in the transected cats. Extended periods of rate variability comparable to those seen during active waking or REM sleep were not observed. Sustained unit rate increases occurred only in conjunction with phasic arousals.

In conclusion: Medullary RF unit recordings, as well as long term monitoring of EMG and respiratory variables, support the conclusion that the chronic medullary cat does not have REM sleep-like states. In these cats, medullary RF units exhibit long periods of very regular discharge, interrupted by brief arousals which recur in a regular ultradian rhythm whose period approximates that of the REM sleep and basic rest-activity cycle.

348.12 BASAL FOREBRAIN UNIT ACTIVITY ACROSS THE SLEEP-WAKING CYCLE IN FREELY MOVING CATS. R. Szymusiak and D. McGinty. Neurophysiol. Res. (151A3), V.A. Medical Center, Sepulveda, CA 91343.

The basal forebrain (BF; preoptic area and surrounding ventral telencephalon) has been implicated in control of the sleep-waking cycle, various homeostatic mechanisms, and in the neuropathology of Alzheimer's disease. The BF has hypnogenic functions; BF electrical stimulation evokes sleep with short latency, and BF lesions suppress sleep. Our first purpose was to determine if any population of BF cells have elevated discharge rates (DR's) during sleep. The BF also has thermoregulatory functions; the area contains thermosensitive neurons, and BF ablations impair thermoregulation. In cats, thermoregulatory effort is greatest during waking, attenuated during non-rapid-eye-movement sleep (nonREMS), and virtually absent during REMS. This has been attributed to corresponding decreases in hypothalamic thermosensitivity. Our second purpose was to determine if any BF cell populations become progressively less active across nonREMS and REMS.

Extracellular action potentials were recorded via chronically implanted microwires (25μ diameter) in freely moving cats. The cats were also implanted for standard sleep recordings. The activity of 40 units in 3 cats has been examined. Two classes of cells were defined. Type 1 cells had higher DR's during waking than during nonREMS (n=33), and Type 2 cells were more active during nonREMS than during waking (n=7).

As a group, DR's of Type 1 cells were not depressed during REMS relative to waking and nonREMS. DR's during phasic REMS (periods of REMS with intense eye movements) were as high as during waking (8.42 ± 1.22 (spikes/sec ± s.e.) versus $8.11 \pm .99$). DR's for quiet REMS were below waking, but were higher than nonREMS rates ($4.26 \pm .71$ versus $2.64 \pm .51$). Only 3 of 33 cells were less active during phasic REMS than waking, yet these cells had higher DR's during REMS than nonREMS. Thus, we do not find a general depression of BF unit activity during REMS.

Type 2 cells had the highest DR's during nonREMS (7.76 ± 1.18), and the lowest during waking ($0.43 \pm .13$) and phasic REMS ($1.52 \pm .63$). Furthermore, DR's during the transition between waking and nonREMS (defined by the presence of periodic spindles in the EEG) approached nonREMS rates ($5.90 \pm .93$). Although Type 2 cells accounted for only 17% of the total sample, they comprised 44% of all cells recorded below horizontal plane -3.0. The localization of these cells to the ventral BF corresponds to sites where electrical stimulation evokes nonREMS, and to areas of the cat BF which stain intensely for choline acetyltransferase. This finding supports the hypothesis of a cholinergic BF hypnogenic system.

Supported by the Veterans Administration

348.13 INTERCOSTAL MUSCLE ACTIVITY (IMA) CORRELATED WITH PONTOGENICULO-OCCIPITAL (PGO) WAVES IN REM SLEEP BEFORE AND AFTER BILATERAL PONTINE-TEGMENTAL LESIONS. T. Dick, P. L. Parmeggiani*, and J. Orem. Department of Physiology, Texas Tech University Health Sciences Center, School of Medicine, Lubbock, TX 79430.

In REM sleep, postural muscles develop an atonia that results from the inhibition of alpha motoneurons and is prevented by bilateral lesions of the dorsal pontine-tegmentum. The intercostal muscles, whose activity has both postural and respiratory components, become hypotonic with variable amounts of respiratory activity persisting during REM sleep. Reports have shown that diaphragmatic and medullary respiratory unit activity changes in association with PGO waves. We studied the relationship of IMA to PGO activity before and after bilateral pontine-tegmental lesions (target: P 3.1, L 2.0., H -1.5, -2.5, -3.5).

Five adult cats had electrodes for recording the electroencephalogram, the electrooculogram, PGO waves, and electromyograms from two intercostal muscles. The data obtained from restrained and unrestrained animals were recorded on a polygraph and an analog tape recorder. IMA was half-wave rectified, integrated, and summed over 6.38-s periods. Simultaneously, PGO waves were discriminated and totaled. We correlated IMA with PGO waves throughout the REM sleep period and constructed cross-correlation histograms of IMA for 250 ms before to 250 ms after PGO waves. These analyses were done on data collected before and after the animal was lesioned.

During REM sleep in intact animals, we observed a hypotonia in the intercostal muscles with respiratory related activity persisting. There was no systematic relationship between PGO activity and IMA. Correlation coefficients , between the amount of IMA and the density of PGO activity, varied around zero with the mean "r" equaling -0.3 + 0.26; 24 REM episodes. Similarly, cross-correlation histograms did not reveal consistent increases or decreases in IMA temporally related of a PGO wave.

Pontine lesions produced various disturbances in REM sleep. In some animals, REM sleep did not occur after lesions. Others were roused by violent jerks upon entering REM sleep. A third class had REM sleep, but still showed a decrease in IMA with REM sleep onset; however, they did show an increase in IMA associated with PGO waves. The "r" value (mean "r"=0.37 + 0.19, N=28) shifted to significantly more positive values than in the unlesioned state ($P<0.005$, Student's "t" Test). In some cases, the cross-correlation histograms revealed an inhibition followed by an excitation in IMA after the occurrence of a PGO wave. We conclude that the pontine lesion either interfered with the process generating REM-sleep atonia or altered phasic REM influences to facilitate IMA. (Supported by Tarbox Parkinson's Disease Institute, TTU, and NIH grant HL 21257.)

348.14 THE ORGANIZATION OF BEHAVIORAL STATES DURING THE THIRD TRIMESTER IN THE FETAL LAMB. H.H. Szeto* and D.J. Hinman*. (SPON: E.D. Weitzman). Department of Pharmacology, Cornell University Medical College, New York, New York 10021.

Three distinct behavioral states, namely arousal (A), quiet sleep (QS), and rapid eye movement sleep (REMS), have been described in the fetal lamb during the late third trimester (Szeto, Am. J. Ob. Gyn. 146:211-217, 1983). We have now investigated the developmental pattern of behavioral states organization in the fetal lamb during the third trimester.

Fetal behavioral states were determined from chronic intrauterine recordings of fetal ECoG, EOG, nuchal EMG, blood pressure, heart rate, and breathing movements via diaphragmatic EMG. A total of 65 recordings (186 hrs) were obtained from 21 fetal lambs ranging from 114-144 days of gestation (term being 145 days). All fetal blood gases were within normal limits, and 19 of the 21 fetuses delivered normally at term.

Organized behavioral states were readily identified in all fetuses over 121 days, and in 50% of the fetuses between 114-121 days of gestation. The distribution of behavioral states as a percent of total recording time and median episode duration at various gestational ages are summarized in the following table:

Age(days)	<121	121-130	131-140	>140
	Percent of total recording time			
A	11.2±5.2*	11.8±8.0	15.1±9.0	18.3±8.8
QS	35.1±7.6	38.4±7.8	38.1±8.5	43.8±7.5
REMS	52.7±7.3	49.9±8.6	46.1±9.4	37.7±8.4
	Percent of total sleep time			
REMS	60.4±7.6	56.8±8.1	54.3±8.9	46.3±7.3
	Median episode duration (min)			
A	2.7	2.6	3.0	3.0
QS	7.1	7.2	9.1	9.1
REMS	7.3	9.5	11.6	11.3

* mean SD

Our results show that the fetal lamb in the third trimester spends 80-90% of the time in sleep, and that REMS comprises 45-60% of sleep time. Transition from A to REMS occurs frequently in the fetus at all gestational ages. In addition, the length of the sleep cycle increases significantly throughout the third trimester. (Supported in part by NIDA-02475-03 & BRSG SO7-RR-5396.)

348.15 SLEEP IN SEVERELY UNDERNOURISHED INFANTS. E.A.Vivaldi*, I.López*, C. G. Perales*, E. Heresi* and N. Colombo*(SPON: N. Inestrosa). I.N.T.A., U. de Chile.

Sleep of severely undernourished infants (more than 3 S.D. under expected weight for age) (UI), aged 6-9 months, during their second week at a rehabilitation center, and of control infants (CI) at their home, was studied using two somehow complementary methodologies. An 8 mm. movie camera focusing the infant cradle was adapted to take one frame per minute for 4 consecutive days to assess sleep and wakefulness and to detect and classify body motility (N = 5 UI, 5 CI). Two naps close to noon were directly observed to provide a detailed description of sleep with rapid eye movements (REMS), and of non-REM sleep (NREMS), which included timing with a stopwatch the presence of eye movements in 30 second epochs (N = 7 UI, 6 CI).

Time-lapse study showed differences in total sleep time (713±22;617±43 mins.[mean UI±s.e.m.;mean CI±s.e.m.]), especially in the amount of sleep between 08:00 and 20:00 (219±16;138±11); and in longest period of uninterrupted wakefulness (338±38; 435±52). A striking periodicity in body motility was found for the first half of the night in CI, with postural shifts occurrence time-locked to sleep onset at 50 and 100 minutes. Such organization was absent in UI.

Nap observations showed a significantly shorter latency to REMS in UI (24.5±2.1;30.7±1.8) and a larger fraction of total sleep spent in REMS (.200±.017;.153±.014). Duration of first REMS episode did not differ significantly (9.3±.9;8.3±1.1). During NREMS a high fraction of epochs showed slow eye motility in UI (.304±.032;.163±.027), whereas in CI this was confined to the 2.5 minutes immediatly preceding REMS. The most remarkable differences were found in the intensity of REMS signs: for example, the fraction of all REMS epochs where eye movements were present for more than half of the epoch was significantly higher in UI (.155±.025;.030±.013). Time course of REMS signs intensity throughout a REMS episode also differed.

Results are discussed in terms of potential alterations in the ontogeny of brain mechanisms underlying the maturation of sleep and wakefulness. Since UI represent extreme cases of nutritional and environmental deprivation, hypothesis of REMS functional role in providing early endogenous stimulation are particularly relevant.
M.1500-8323, U. de Chile.

348.16 ORGANIZATION OF WAKING AND SLEEP-STATES IN INFANTS: BEYOND PERIODICITY. Evelyn B. Thoman and Christine Acebo*. Department of Biobehavioral Sciences, University of Connecticut, Storrs, CT 06268.

Behavioral states of wakefulness and of sleep are sensitive indicators of CNS functioning. We have made continuous seven-hour observations of infants in their homes in order to describe the full range of naturally occurring sleeping and waking states during the early weeks of life. The states observed include: alert, waking (non-alert) activity, fussing, crying, drowse, sleep-wake transition, active sleep, active-quiet transition sleep, and quiet sleep. From observations of infants at 2, 3, 4, and 5 weeks of age, it was determined that the amount of time spent in each of these states reliably characterizes individual infants. Organization of these states during the portion of the day when the infants were not with the mother were depicted by a profile of percentages of baby-alone time allocated to each state. The consistency of profiles over the four-week period was assessed using an analysis of variance procedure, yielding a descriptive F score, a state stability index. In a group of 28 infants, these F values ranged from 3.1 to 304.9, and those with very low scores were found to show developmental dysfunction at a later age. The sequential occurrence of states throughout each observation day was also depicted for each subject. The stability score is independent of periodicities found for the sleep states. It can be concluded that the stability index reflects systematic, temporal constraints that link the states of wakefulness and sleep, and these constraints are independent of periodicity as typically assessed.

348.18 SLEEP DEPRIVATION INCREASES SUBSEQUENT NOCTURNAL hPRL SECRETION.
I. Feinberg, D. Kleinberg*, S. Rothenberg*, C. Hollander*.
VA Medical Center and NYU Bellevue, New York and VA Medical Center and University of California, San Francisco.

Human prolactin (hPRL) secretion is elevated during sleep (Weitzman, E.D., Ann. Rev. Med. 27:225, 1976). A report (Parker et al., J. Clin. End. Metab., 38:646, 1974) that secretory peaks coincide with NREM periods has not been substantiated (see Van Cauter, E. et al., ibid., 54:70, 1982). Nevertheless, the functional significance of the nocturnal elevations of hPRL secretion is unknown and it remains possible that this hormone plays some role in sleep processes. Our study had two goals: (a) to determine whether hPRL secretion varies according to sleep need, and (b) to assess further the relation of hPRL secretory peaks to the cyclic phenomena of sleep including peaks of delta wave activity as measured by computer (Feinberg, I. et al., Electroenceph. Clin. Neurophysiol., 44:202, 1978). Subjects (Ss) were 12 normal adult males aged 18-27 yrs.; mean=23.4 yrs. Blood sampling and EEG-EOG recording were carried out on a single recording night (RN) in the Clinical Research Center at Bellevue Hospital. Sleep need was manipulated by varying the amount of sleep on the night preceding (NP) the RN. The three conditions on the NP were: 0h in bed (i.e., total sleep deprivation); 8h in bed (baseline sleep); and 12h in bed (extended sleep). Bedtimes were at 11:00PM each night. Two weeks separated conditions. Although EEG recording was not carried out on the NP, data from other studies (Feinberg, I. et al., ibid., 49:467, 1980) allow us to estimate the waking durations preceding the RN as: 40h, 16h, and 13.5h, for the 0h, 8h, and 12h conditions, respectively. Blood samples were drawn at 10-min intervals beginning at lights out and throughout the sleep period. Two to three waking samples were taken prior to lights out and after final awakening. Samples were placed in an ice bath, spun down at 0C within 50 min, and the plasma frozen and stored at -20C. The RIAs were carried out "blind" on coded samples at the NYVA according to methods previously described. The data from all conditions of a single subject were analysed in duplicate in a single assay. This report is based on hPRL data from 9 Ss, of whom 7 completed all three conditions and 2, two conditions. Mean hPRL after SD was 10.80ng/mL this value was significantly higher ($F(1,13)=5.43$; $p<.05$) than the values of 8.92 and 8.85 for the 12h and 8h conditions, respectively. The size of this effect thus appears modest. To evaluate the periodicity of hPRL secretion while controlling for individual differences in level, each S's data were converted into z-scores using his mean and SD across all conditions. Smoothed plots of these scores revealed a large early peak followed by 1-3 later smaller ones. This pattern appears similar to that described by Van Cauter et al. (idem). The relation of these peaks to sleep cycles and delta wave peaks will be the subject of future analyses.

348.PO ATTENTIVE WAKEFULNESS AND SLEEP: INVERSE DEVIATIONS FROM RANDOM MODEL OF NEURONAL FIRING PATTERNS IN THE FELINE CENTRUM MEDIANUM,
T. J. Marczynski and L. L. Burns*. Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago, IL 60612.

In 15 freely moving cats, bearing electrodes in the centrum medianum-parafascicular complex (CM-Pf) of the thalamus, temporal patterns of single neuronal spike trains were analyzed during attentive wakefulness (AW) and slow wave sleep (SWS). The patterns were analyzed using a non-parametric method based on relative relations between the sequential spike intervals. The magnitudes and the direction of deviation of patterns from the random model were quantified using chi square statistics (Marczynski et al. Brain Res., 185:139,1980).- Upon behavioral transition from AW to SWS, 13 of 33 neurons studied showed significant inverse pattern distributions with reference to the random model: patterns strongly emitted during AW subsequently occurred much less often during SWS than predicted by the random model or were virtually eliminated from the otherwise very active firing repertoire. Conversely, patterns that failed to occur during AW or occurred much less often than expected became dominant during SWS. Most importantly, the emission magnitudes of individual patterns during AW were positively correlated with their subsequent deficit magnitudes during SWS. This indicates that the statistical inversions in the distribution of patterns were homeostatically regulated. The well-known graded nature of receptor desensitization to specific neurotransmitters seems to offer a plausible explanation for the correlation between the emission magnitudes of patterns and their subsequent deficits below chance level during SWS. The emergence of SWS firing patterns, i.e. those that were virtually absent during AW, indicates that the intact and "unused" during AW receptor patches at the soma-dendrites became active during SWS. Similar statistical inversions of pattern distributions have been observed in spike trains from the nuc. reticularis thalami in freely moving cats upon transition from bar pressing performance to SWS (Marczynski, T.J. et al. Experientia, in press). In the CNS, axonal contacts mediated by different tansmitters are not randomly distributed on the soma-dendrites but are segregated to specific dendritic regions (Hoffert, J. et al.,Soc. Neurosci.,Abstracts, 8(2):805,1982). We suggest that the receptor patches of CM-Pf neurons responsible for statistical inversions of AW and SWS firing patterns have complementary spatial distribution. Therefore, if selectively activated during AW and SWS, trigger sets of patterns whose statistical distributions are inversely related to one another and complementary. Such relationships may be essential for the recuperative function of SWS in view of the reports that depolarizing impulses applied to brain tissue markedly enhance receptor resensitization (Wagner, H.R. and Davis,J.N., Proc. Natl. Acad. Sci. USA, 76:2057, 1979).- Supported by PHS MH 8396.

PEPTIDES: RECEPTORS III

349.1 AUTORADIOGRAPHIC LOCALIZATION OF [^{3}H]-ARGININE VASOPRESSIN BINDING SITES IN THE RAT BRAIN AND KIDNEY.
F.W.van Leeuwen*(SPON: European Neuroscience Association). Netherlands Institute for Brain Research, Dept. of Brain-Endocrine Interactions, IJdijk 28, 1095 KJ Amsterdam, The Netherlands.

Recently it has become clear that vasopressin (VP) containing cell bodies are distributed over at least 7 cell groups in the rat brain, projecting widely but selectively throughout the brain (Van Leeuwen and Caffé, Cell and Tiss. Res. *228*, 525, 1983; De Vries and Buijs, Brain Res., in press). VP meets many of the criteria which must be fulfilled before it can be considered as a proven neurotransmitter (cf. Buijs, Prog. Brain Res., *55:* 167, 1982). One of the criteria which has not yet been met is that specific receptors interact with the substance in question, and do so in close proximity to synaptic structures.

In order to reveal VP binding sites, the methodology developed by Kuhar c.s. was followed using [^{3}H]-Arg VP (NEN, 45.3 Ci/mmol) and tritium sensitive Ultrofilm (e.g. Wamsley, J. Histochem.Cyt.*29*, 125, 1981). The rat kidney served as a control. Prior to autoradiography, initial binding experiments revealed specific but inconsistent binding especially in the brain (septal level). Requirements for improving the ratio between total and non-specific binding are now under investigation.

Nevertheless, after incubation with 5nM [^{3}H] VP, autoradiography revealed grains both in the cortex (distal convolute) and even more so in the medulla (collecting ducts) of the kidney. In brain sections especially the lateral septum showed high grain densities. Virtually no grains were seen in consecutive sections treated in identical fashion except for the addition of 5 µM unlabelled VP, although other areas (e.g. the hippocampus) showed high aspecific labelling. The binding sites in the lateral septum show an almost perfect overlap with the VP fibres which terminate synaptically within this area. In conclusion, the presence of VP binding sites in the lateral septum adds further support for the hypothesis that VP acts as a neurotransmitter within the brain.

349.2 CHARACTERIZATION OF OXYTOCIN RECEPTORS IN THE RAT HIPPOCAMPUS AND BRAINSTEM.
M. Mühlethaler* and J.J. Dreifuss, Dept. Physiol., Univ. Med. Ctr., Geneva, 1211 Switzerland.

Arginine vasopressin (VP) and oxytocin (OT) increase the rate of firing of a class of neurones in hippocampal slices. In the present study, we attempted to assess whether receptors for neurohypophysial peptides are similar in such widely different parts of the body as the brain and peripheral tissues responsive to these hormones. To achieve this end, we first compared systematically the effects of OT and VP. Each cell recorded from was exposed to VP at 1 µM and to at least two further concentrations of VP or OT applied in random order. We computed in each instance the increase in firing rate from resting to peak level reached in presence of the peptide. The lowest effective concentration of OT was 1 nM and stimulation was maximal at 1 µM. The two dose-response curves were found to run in parallel, VP producing a 10-30 times smaller increase in firing than OT when applied at the same concentration. Since this greater sensitivity to OT was noticed for every cell tested, we assume that OT and VP act on a homogenous population of cells.

Next we used a number of derivatives, natural or synthetic, possessing known endocrine effects. These compounds included arginine vasotocin, as well as four synthetic analogues. The potency of each compound was determined by applying it at 1 µM for 5 min and by counting the total number of action potentials generated in response to peptide application. To facilitate comparison, the potency of each compound was expressed relative to that of VP also applied at 1 µM in every experiment. The relative potency in the hippocampus of OT, VP and all five analogues correlated well with their oxytocic activity, whilst it bore no apparent relationship with either their vasopressor or antidiuretic activities. Moreover, amongst the analogues, a selective oxytocic agonist had a powerful effect in the hippocampus, while an analogue acting as an antagonist in the uterus blocked the response to OT. Taken together with immunohistochemical data indicating the presence in the rat hippocampus of OT-containing axons, these data suggest that an excitatory effect of neurohypophysial nonapeptides in the hippocampus might result from an interaction with central receptors similar to the receptors present in the uterus.

Preliminary studies showed that neurones in the dorsal motor nucleus of the vagus nerve are similarly excited by various neurohypophysial peptides, suggesting that oxytocic receptors may have a widespread distribution in the mammalian brain.
(Supported by Swiss NSF grant 3.875.081).

349.3 AUTORADIOGRAPHIC LOCALIZATION OF VASOPRESSIN BINDING IN THE RAT BRAIN. F.M. Petracca*, D.G. Baskin*, and D.M. Dorsa*.(Spon: D. M. Bowden). Departments of Pharmacology, Psychology, Biological Structure, and Medicine, University of Washington, Seattle, WA 98195, and the Veteran's Administration, Seattle, WA 98108.

Arginine vasopressin (AVP) has long been known to act as an antidiuretic hormone and as a pressor agent. Recent anatomic, pharmacologic, and behavioral evidence suggest that AVP may serve as a CNS neurotransmitter or neuromodulator. Vasopressinergic neurons originating in the magnocellular nuclei of the hypothalamus have been shown to project to many brain regions, including the septum, thalamus, amygdala, and nuclei such as the locus coeruleus and the nucleus of the solitary tract. Local injections of AVP into several of these discrete areas result in effects on central regulation of temperature and blood pressure, as well as on behavior. This evidence suggests that there are target neurons with vasopressin receptors within the brain. In the present study, binding of ^{3}H-labeled AVP to brain slices was used to localize putative CNS vasopressin receptors.

Conditions for the binding assay were optimized using kidney tissue. Binding of ^{3}H-AVP to kidney slices was saturable, reversible, and specific for AVP. Autoradiographic localization of vasopressin binding was restricted to kidney medullary tissue. Binding of ^{3}H-AVP to brain slices was performed using the same assay procedure. 20-micron thick sections of rat brain were incubated in 100 microliters of 5 nM ^{3}H-AVP (S.A.=42.1 Ci/mmol, New England Nuclear) in Tris buffer, in the presence or absence of 1 μM unlabeled AVP. Specific binding as high as 31% was observed in these brain sections. The anatomical distribution of AVP specific binding was examined using tritium-sensitive film. The distribution of radioactivity on the brain sections suggested that AVP binding sites are concentrated in discrete foci. Specific ^{3}H-AVP binding was observed in the lateral septum, amygdala, ventral thalamus, and nucleus of the solitary tract.

The localization of AVP specific binding in these areas, which are known to receive vasopressinergic innervation, and which have been implicated as central sites of action of vasopressin, supports the hypothesis that the CNS is a target organ for AVP and provides evidence for AVP receptors which might mediate its effects.

349.4 **LIGHT MICROSCOPIC AUTORADIOGRAPHIC DISTRIBUTION OF VASOPRESSIN BINDING SITES IN RAT CENTRAL NERVOUS SYSTEM.** R. E. Brinton, K. W. Gee, J. K. Wamsley+ and H. I. Yamamura. Dept. of Pharmacology, Arizona Health Sciences Center, University of Arizona, Tucson, AZ 85724 and +Dept. of Psychiatry, University of Utah Medical Center, Salt Lake City, UT 84132.

Vasopressin (AVP) has been the focus of intensive study to elucidate its functional role in the CNS. Immunocytochemical and radioimmunological studies have indicated intracellular and synaptic localization within the hypothalamus while only the latter occurs in extrahypothalamic brain regions. Electrophysiological studies suggest that AVP acts as a neuromodulator. In light of these biochemical and physiological findings, the profound effect of this neuropeptide on memory processes has been especially interesting and is suggestive of specific CNS receptors for AVP.

Male Sprague Dawley rats (250-300 g) were sacrificed, brains immediately removed and placed on ice. Each brain was prepared for microtome sectioning by coating the brain in plastic embedding medium and freezing it onto a microtome chuck by immersing in liquid nitrogen. Preparation of brain sections for light microscopic autoradiography followed the method of Wamsley and Palacios, 1983. Briefly, 10 um coronal sections were cut from whole rat brain and thaw-mounted on chrome alum/gelatine-coated slides. Slide mounted sections were stored overnight at 0 deg. C. Each section was thawed and preincubated in ice-cold buffer for 20 min prior to labeling with tritiated AVP (45.3 Ci/mM, NEN). Paired sections were incubated with 5 nM tritiated AVP in the presence or absence of 1 uM unlabeled AVP at 0 deg. C for 60 min. Incubations were terminated by rinsing each section in ice-cold buffer followed by a final rinse in distilled water. Autoradiographs were prepared by exposing slide-mounted sections to tritium-sensitive film for 1 week.

Measurements of optical density from autoradiographs showed discrete areas of high grain density in intrahypothalamic areas. Dense labeling of tritiated AVP binding sites were observed in the supraoptic, paraventricular and suprachiasmatic nuclei. Within the paraventricular nucleus, the magnocellular region was labeled to a greater degree than the parvocellular region. Dense labeling was also observed in the arcuate nucleus and the median eminence-tubroinfundibular region. Low levels of tritiated AVP binding sites were seen in the hippocampus and in the substantia nigra. Within the hippocampus CA1 and the granule cells of the dentate gyrus were labeled. These binding sites, which may represent specific CNS receptors for AVP, are being further characterized.

(Supported by NIMH Predoctoral fellowship MH-08941 to REB and USPHS-RSDA MH00095 to HIY.)

349.5 THE DISTRIBUTION AND CHARACTERISATION OF SUBSTANCE P RECEPTORS IN THE CENTRAL NERVOUS SYSTEM P.W. Mantyh*, R. Pinnock*, P. Downes*, B. Sandberg*, M. Goedert* and S.P. Hunt*. (SPON: Michael C. Kennedy) MRC Neurochemical Pharmacology Unit, Medical Research Council Centre, Medical School, Hills Road, Cambridge CB2 2QH, UK.

We have used a battery of techniques in an attempt to map the distribution of substance P (SP) receptors within the central nervous system. Tritiated SP (^{3}H-SP) was found to bind to rat and bovine tissue in a saturable fashion with a K_D of approximately 1-2 nM. The relative potencies of SP free acid, SP^{5-11}, SP^{6-11} and SP^{1-9} in displacing ^{3}H-SP from tissue sections was comparable to that observed in the guinea pig ileum suggesting that binding was taking place to a functionally important receptor. Using autoradiography coupled with LKB tritium sensitive Ultrafilm or the dry emulsion coated coverslip technique the distribution of ^{3}H-SP binding sites was found to be highest within the olfactory bulb, amygdala, hypothalamus, locus coeruleus and the solitary nucleus. It is moderate in the olfactory tubercle, frontal cortex, striatum, central grey, parabrachial nucleus, and moderate to low in the hippocampus, cerebellum, trigeminal nucleus pars caudalis and spinal cord. Interestingly, very low levels of receptors were observed in several regions where high levels of SP immunoreactivity have been reported such as the substantia nigra pars reticulata. Additional evidence that these ^{3}H-SP binding sites were peptide receptors came from studies of phosphatidylinositol (PI) turnover. Vibratome slices from discrete areas of rat brain described above, judged to be living by electrophysiological parameters, were stimulated by 1 uM SP in the presence of ^{3}H-inositol. In general the rate of hydrolysis of inositol phospholipids was proportional to the concentration of ^{3}H-SP receptors we have previously measured using LKB film coupled with densitometry in these areas.

Taken together these results suggest: 1) SP receptors are located in discrete areas of the rat and bovine brain; 2) these SP receptors appear to be functionally active; 3) that the levels of SP receptors are not obviously correlated with those regions that display the highest levels of immunoreactive SP.

349.6 **DISTRIBUTION OF ^{3}H-SUBSTANCE P BINDING SITES IN CNS AND PERIPHERAL TISSUES OF THE RAT AND THE GUINEA PIG.** S.H. Buck, T.F. Burks and H.I. Yamamura. Department of Pharmacology, University of Arizona College of Medicine, Tucson, Arizona 85724.

The undecapeptide, substance P (SP), is thought to be a neurotransmitter/neuromodulator in the mammalian CNS and peripheral nervous system. Immunohistochemical and radioimmunoassay studies have indicated marked differences in numbers of SP-containing neurons in certain tissues in the rat compared to the same tissues in the guinea pig; i.e., guinea pigs have 3 to 5-fold higher SP levels in sensory neurons and 5 to 10-fold higher levels in the gastrointestinal tract compared to rats. We have used ^{3}H-SP to compare the amount of high affinity binding in these and other tissues of the two species.

Crude membranes from various tissues were prepared and incubated with slight modification of the methods of Lee & Snyder (Mol. Pharm., in press) and Quirion et al. (Nature, in press). Tissues were homogenized in 20 mM tricine (pH 7.4) containing 120 mM NaCl and 5 mM KCl, resuspended in 20 mM tricine (pH 7.4) containing 300 mM KCl and 10 mM EDTA for 1 hr at 4°C, and resuspended and washed two times in plain 20 mM tricine (pH 7.4). Membranes (4% final tissue concentration) were then incubated for 20 min at 20°C in 20 mM tricine (pH 7.4) containing BSA, bacitracin, chymostatin, leupeptin, 3 mM $MnCl_2$, and 2 nM ^{3}H-SP, followed by filtration. Specific binding was defined as the difference between the absence and presence of 1 µM unlabeled SP. Under these conditions, specific binding was linear with tissue, saturable at ≈ 10 nM ligand, and constituted 50% (spinal cord) to 95% (submandibular gland) of total binding. The K_D was ≈ 1 nM in most tissues.

^{3}H-SP Binding (pmole/g tissue)	RAT	GUINEA PIG
Submandibular gland	6.5 ± 0.5	2.8 ± 1.0
Intestinal long. muscle	0.9 ± 0.3	1.2 ± 0.2
Hypothalamus	1.0 ± 0.2	0.4 ± 0.1
Hippocampus	1.4 ± 0.1	0.4 ± 0.0
Corpus striatum	1.3 ± 0.2	1.0 ± 0.2
Dorsal spinal cord	0.3 ± 0.1	0.2 ± 0.0
Ventral spinal cord	0.2 ± 0.0	0.1 ± 0.0

These results indicate that there are distinct differences in amount of high-affinity SP binding in the rat and the guinea pig, especially in brain regions and in salivary gland. Moreover, they indicate that marked differences between the two species in SP levels in a tissue are not necessarily reflected by marked differences in amount of binding sites (e.g., intestinal longitudinal muscle). In addition, our results support the observations of Quirion et al. (op.cit.) that within a species the relative amount of SP binding does not necessarily correlate with the relative SP levels in tissues (e.g. hippocampus vs. corpus striatum in rat).

Supported by USPHS Grants DA-02163 and MH-27257.

349.7 **POTENCY DIFFERENCES BETWEEN SUBSTANCE P (SP) AND Tyr^0, Nle^{11}-SP (TNSP) IN NEURONAL ASSAYS, BUT NOT IN THE GUINEA PIG ISOLATED ILEUM (GPI).** F. Porreca, S.H. Buck, P.S. Darman*, T.F. Burks, H.I. Yamamura & V.J. Hruby*, Departments of Pharmacology, Biochemistry & Chemistry, University of Arizona, Tucson, AZ 85724

It has previously been suggested that the SP receptors in the GPI may differ from those in other tissues (Hawcock et al., Eur. J. Pharmacol. 80:135). We addressed this issue by comparing the potency of SP and a novel analog, TNSP, in a SP effect primarily believed to be myogenic (contractions of GPI) and in several neural SP assays: the production of caudally-directed biting attempts after intrathecal (i.t.) injection to mice, and the displacement of specific ^{3}H-SP binding from rat brain, rat salivary gland and GPI longitudinal muscle-myenteric plexus (LM-MP).

Strips of GPI were suspended in Tyrode's and exposed to graded concentrations of SP or TNSP. Groups of 5-8 mice received graded i.t. doses of SP or TNSP (delivered by direct lumbar puncture under light ether anesthesia) and caudally-directed biting attempts were counted for 1 min, 3 min after injection. Competition of SP or TNSP for binding of ^{3}H-SP (2 nM) to crude membranes of rat brain, rat salivary gland and GPI LM-MP was carried out as described by Buck et. al., in these proceedings. Specific binding was defined as the difference in binding in the presence and absence of 1 µM cold SP and was about 80-90% of total binding. SP binding in GPI LM-MP produced a curvilinear Scatchard plot suggesting a small number of high affinity and a large number of low affinity sites.

Both SP and TNSP were equiefficacious and equipotent in producing contractions of the GPI (A_{50} = 3 and 2.4 nM, respectively). In contrast, TNSP was 4 times less potent in producing biting attempts after i.t. injection to mice, 7.5 times less potent in displacing ^{3}H-SP from rat brain or rat salivary gland, and about 11 times less potent in displacing ^{3}H-SP from GPI LM-MP than SP. The affinity of SP in binding to rat brain, rat salivary gland and GPI LM-MP suggested activity at a high affinity site ($K_D \cong 1$ nM). These results suggest that (a) the receptors to which ^{3}H-SP binds in the rat brain are the same as those in the rat salivary gland and (b) that these sites are probably analogous to those activated in the mouse spinal cord following i.t. injection. In addition, the IC_{50} values and differences in potency between SP and TNSP in competing with ^{3}H-SP binding in GPI LM-MP suggest that binding is preferentially occurring at a high affinity (neural?) site in this tissue. Since SP and TNSP are equipotent in producing contractions of the GPI, a separate (myogenic?) receptor, different from that in neural SP assays, may be involved in this effect. In this regard, SP contractions of the GPI are primarily atropine-resistant. Supported by DA 02163 and MH 27257.

349.8 SPECIFIC BINDING OF [^{3}H]SUBSTANCE-P TO THE RAT SUBMAXILLARY GLAND. S.W. Bahouth* and J.M. Musacchio. Dept. of Pharmacology, New York Univ. Med. Ctr., New York, NY 10016.

The putative neurotransmitter substance-P (SP) has numerous central and peripheral biological effects, including salivation. We have found a specific, saturable and high affinity binding site for [^{3}H]SP in rat submaxillary gland homogenate.

Rat submaxillary glands were homogenized in 20 mM HEPES pH 7.4 and centrifuged at 37,000 g. The pellet was resuspended in 20 mM HEPES, and then incubated at 20°C for 15 min with 0.1 mM TLCK and TPCK, to inhibit trypsin and chymotrypsin like enzymes. After cooling, the homogenate was centrifuged at 37,000 g. The pellet was resuspended at a 1:35 dilution in the binding medium composed of 0.2 M sodium sulfate, 20 mM HEPES pH 7.4, 0.05 mg/ml chymostatin and 0.3 mg/ml bacitracin. The tissue homogenate (0.4 ml) was incubated with increasing concentrations of [^{3}H]SP (0.2-20 nM) in a total volume of 0.5 ml, at 20°C for 20 min. The incubation was terminated by cooling on ice for 2 min then filtered through Whatman GF/B filters pretreated, for at least four hours, with 0.05% polyethylenimine in water.

The binding of [^{3}H]SP and its displacement by SP and physalaemin were sensitive to the ionic strength of the binding medium. In low ionic strength, i.e. 0.3 M sucrose, 20 mM HEPES pH 7.4, [^{3}H]SP binds to two binding sites, a high affinity site with K_D 0.15 nM and B_{max} 16.2 p mol/gm tissue and a low affinity site with K_D 65.7 nM and B_{max} 104 ± 49 p mol/gm tissue. The Hill slope for the high affinity site was 0.72. The ability of physalaemin to displace 4 nM [^{3}H]SP was very weak compared to SP, IC_{50} 200 ± 50 nM versus 4.63 ± 1.9 nM for SP. This finding was unexpected because physalaemin is twice as potent as SP as a sialogogue.

In the presence of high ionic strength (µ) media, the ability of physalaemin to displace [^{3}H]SP improved dramatically. In 0.2 M sodium sulfate (µ=0.6) physalaemin has an IC_{50} of 5.6 ± 0.32 nM versus 6.65 ± 1.7 nM for SP. Scatchard analysis of the saturation experiments showed that [^{3}H]SP was binding to a single high affinity site K_D 2.8 ± 0.5 nM and B_{max} 16.4 ± 2.5 p mol/gm tissue or 270 ± 30 f mol/mg protein with a Hill slope of unity. The relative displacement potency of various SP analogs and fragments correlated with their relative salivation potency in rats. None of the nucleotides tested (in 0.2 M sodium sulfate) was an effective inhibitor of [^{3}H]SP binding, their IC_{50} was in the millimolar range. In the same binding medium Mn^{++} and Mg^{++} (5 mM) increased the binding of 4 nM [^{3}H]SP by 35 ± 5 percent. This work was supported in part by PHS grants DA02013, MH29591 and MH17785 to J.M.M.

349.9 DEVELOPMENTAL REGULATION OF SUBSTANCE P RECEPTORS BY SUBSTANCE P. G.E. Handelmann*, C. Helke, T. O'Donohue, C. Shults*, and T.N. Chase. Lab. Cell Biology, NIMH, Bethesda, Md.; Dept. Pharmacology, USUHS, Bethesda, Md.; Experimental Therapeutics Branch, NINCDS, Bethesda, Md. 20205 (SPON: J. Newman).

A number of hormones play a role in the development of their target organs. The present experiments demonstrate that a neuropeptide hormone, Substance P (SP), has a developmental influence on its targets, specifically with respect to their adult sensitivity to SP, and that the altered sensitivity is most likely due to a change in SP receptors.

Neonatal albino rats received subcutaneous injections of SP (1 ug/pup) or saline daily on days 1-7 after birth. When the rats were at least 90 days old, the function of SP systems was investigated by measuring pain threshold, and salivation and blood pressure in response to intravenous SP. Rats treated as neonates with SP showed a more rapid sensitization to pain in a conventional "paw-lick" test. They also showed a greater sialogogic response to SP, although their hypotensive response was similar to that of the controls.

These results suggested that neonatal administration of SP had a long-lasting effect on pain perception and salivation, and that the rats might be more sensitive than normal to the effects of SP. To begin investigating the possibility that SP receptors were altered, autoradiographic localization of iodinated SP binding to brain slices was performed on the rats used in the previous experiments.

The autoradiographs of rats treated as neonates with SP showed higher optical densities than controls in several brain regions, as follows: 1. central grey, 13%; 2. dorsal raphe, 31%; 3. septo-fimbrial nucleus, 49%; 4. dorsal tegmental nucleus, 57%; 5. locus coeruleus, 196%. These increases may indicate an increase in receptor number or affinity. We are presently making similar measurements in spinal cord and parotid glands.

These experiments demonstrate that early exposure to high levels of SP influences the sensitivity of the adult to SP. The SP treatment increases the amount of specific binding of SP to target tissue. This alteration has physiological significance for the animal, in pain perception and salivation.

349.10 AUTORADIOGRAPHIC VISUALIZATION OF SUBSTANCE P RECEPTORS IN RAT BRAIN. C.B. Pert[1], R.B. Rothman*[1], M. Herkenham[2], M.A. Cascieri*[3], and T. Liang*[3]. (SPON: K. Chang) [1]Sec.on Brain Biochem., NSB, and [2]Lab. of Neurophysiol., NIMH, Bethesda, MD 20202. [3]Merck, Sharp and Dohme Res. Lab., Rahway, NJ 07065.

Lack of a biologically active radiolabeled ligand and failure to optimize preincubation/incubation conditions had previously hindered the reproducible characterization of substance P receptors (SPRs).

Slide-mounted sections of rat caudate (Herkenham and Pert, J. Neurosci. 2:1129, 1982) were preincubated for 30 min at room temp (RT) in a Krebs/TRIS buffer containing 35 µg/ml veratrine and 200 µM GTP, washed for 10 min in 50 mM TRIS, pH 7.4, and dried under a stream of cool air. One ml of incubation medium (50 mM NH_4Ac, pH 7.4, 5 mM $MnCl_2$, 50 ug/ml chymostatin, 1 mg/ml BSA) containing Bolton-Hunter produced ^{125}I-BH-SP (0.1 nM) (Liang and Cascieri BBRC 96:1793, 1980) was applied to the sections. At steady state (90 min/RT), sections were washed by 4 1-min immersions in ice-cold buffer. Nonspecific binding was determined by incubations with 1 µM SP. Specific binding was 95% of total binding. For visualization, the ligand was fixed (95% efficacy) using hot formaldehyde vapors (Herkenham and Pert, ibid). The structure-activity profile of the caudate ^{125}I-BH-SP binding site correlated well (r^2 = 0.91) with SPRs characterized in cortical membranes (Cascieri and Liang, JBC, in press). The order of potency of SP analogues was SP > SP(2-11) > physalaemin > SP(4-11) > SP(3-11) > D-Ala^9-SP(5-11) > SP-ME > eledoisin > D-Pro^2-Try^{7,9}-SP > SP-FA.

SPRs are widely but discretely distributed in the CNS. Densely labeled primary and secondary sensory nuclei include the subfornical organ, superficial layer of the superior colliculus, suprachiasmatic n, n of the solitary tract, external plexiform layer of the olfactory bulb, inferior colliculus, medial parabrachial n and upper layers of sensory cortex. Motor-related areas with SPRs include upper layers of motor cortex, striatum, discrete alternating bands in the molecular layer of folia IX and X of cerebellar vermis, the principal and medial n of the interior olive and n ambiguus. Limbic and other structures with moderate to dense labeling include prelimbic, sulcal, and entorhinal cortex, hippocampus, n. of the diagonal band, medial and central n. of the amygdala, medial habenula, and the locus coeruleus. The substantia nigra and interpeduncular n., which are enriched with SP nerve terminals, had no detectable receptors. Failure to find SPRs in substantia nigra or interpeduncular nucleus, regions highly enriched in SPR terminals, suggests the existence of qualitatively different (e.g., low affinity) SPRs requiring another ligand or different assay conditions for visualization at these loci. The widespread autoradiographic distribution of SPRs, like those for several other neuropeptides visualized to date, suggests a modulatory influence on multiple behavioral functions.

349.11 ^{3}H-Substance P binding sites in rat brain and submaxillary gland. M.H. Perrone*, R.E. Diehl*, D.M. LoPresti*, D. Kiefer*, and D.R. Haubrich (SPON.: D. Luttinger). Central Nervous System Section, Dept. of Pharmacology, Sterling-Winthrop Res. Inst., Rensselaer, NY 12144

Substance P (SP), an undecapeptide, is a neurotransmitter in the mammalian central nervous system. Labeling of putative SP receptors with ^{125}I-Bolton Hunter-conjugated SP has been demonstrated in membranes derived from the submaxillary gland and in primary cultures of mouse mesencephalon. ^{3}H-SP has been used to identify SP receptors in the submaxillary gland by Lee and Snyder (Mol. Pharm. in press) and by Hanley et. al., (Nature 286, 1980) although the latter method had relatively high amounts of non-specific binding. In this report, we compare ^{3}H-SP binding in the submaxillary gland to that in brain tissue in which the non-specific binding has been markedly reduced. A crude membrane preparation (48,000 x g pellet) from whole brain minus cortex and cerebellum was obtained and resuspended in 50 mM TRIS (pH 7.7 25°) 2 mM $CaCl_2$, 2 mM $MgCl_2$, 2 µg/ml chymostatin, 40 µg/ml bacitracin, 4 µg/ml leupeptin, and 200 µg/ml BSA. Submaxillary glands were homogenized and incubated with 300 mM KCl, 120 mM NaCl, and 10 mM EDTA. After three centrifugations at 48,000 x g with intermediate resuspensions in 50 mM TRIS buffer, the membranes were resuspended in the buffer described above without the $CaCl_2$ and $MgCl_2$. Binding was determined at 20° and was terminated by rapid filtration through GF/F filters that had been soaked with 0.1% polyethylenimine. Binding was saturable and reversible. Scatchard analysis of equilibrium binding data revealed linear plots indicative of single-site reactions for both tissues. The calculated K_D and B_{max} for brain tissue were 1 nM and 125 fmoles/mg protein. The respective values for the submaxillary gland were 0.8 nM and 220 fmoles mg/protein. Specific binding, defined as that displaced by 1 µM SP, was 85% of the total at 1 nM ^{3}H-SP. Inhibition of specific ^{3}H-SP (1 nM) binding by SP related compounds is shown below:

Compound	Rat Brain IC_{50} (nM)	Submaxillary IC_{50} (nM)
SP	2.0	1.8
Physalaemin	7.5	7.3
SP_{2-11}	48.1	8
pGlu SP6-11	230	585
Eledoisin	1010	308
D-Pro,^{2} D-Phe,^{7}D-Trp^{9} SP_{1-11}	1444	3000
Kassinin	10500	3.4

These results suggest the SP binding sites in the brain and submaxillary gland differ slightly with respect to affinity and selectivity for SP analogues.

349.12 AUTORADIOGRAPHIC DISTRIBUTION OF SUBSTANCE P RECEPTORS IN THE RAT MEDULLA: EFFECT OF VAGOTOMY AND NODOSE GANGLIONECTOMY. C.J. Helke, C.W. Shults*, T.L. O'Donohue and T.N. Chase. Dept of Pharmacology, Uniformed Services Univ. of the Health Sciences, Bethesda, MD 20814 and Expt. Ther. Branch, NINCDS, Bethesda, MD 20205.

The neuropeptide, substance P (SP), is present in nuclei in the medulla oblongata associated with autonomic and respiratory function. In this study we investigated the distribution and denervation-induced changes of SP receptors in rat medulla with in vitro autoradiography. Slide-mounted, dessicated, 20 µm coronal sections of medulla were incubated with either ^{125}I-Bolton-Hunter SP (Peptides 3: 1073, 1982) or ^{3}H-SP (Nature, in press). LKB Ultrofilm ^{3}H was exposed for either 3d (^{125}I-SP) or 4 wks (^{3}H-SP). Adjacent sections were stained with thionine for histological verification of binding sites.

SP binding sites were found to be widely distributed in the medulla. The highest density was found in the nucleus tractus solitarius (NTS), rostral nucleus ambiguus, dorsal motor nucleus of the vagus, hypoglossal nucleus, spinal trigeminal nucleus and inferior olive. Moderate binding was apparent in the commissural NTS, area postrema, nucleus reticularis parvocellularis, medial vestibular nucleus and facial nucleus. The remainder of the medullary nuclei contained very low or no SP binding sites. Similar results as to the distribution and relative density of binding sites were obtained with ^{125}I-SP as with ^{3}H-SP. Addition of unlabeled SP (1 µm) to the incubation medium prevented regionally distributed binding.

The SP binding sites in the medulla of sham operated rats were compared with those of unilateral nodose ganglionectomized (NGX) or cervical vagotomized (VAG) rats. Computerized densitometry of ^{125}I-SP labeled autoradiograms was used to quantitate denervation-induced changes in the amount of SP binding. ^{125}I-SP binding was unilaterally reduced in the rostral nucleus ambiguus (44%) and the rostral dorsal motor nucleus of the vagus (32%) with either NGX and VAG. No changes in ^{125}I-SP binding were detected in other medullary nuclei. Because both NGX and VAG involve sectioning the efferent vagus nerve, the denervation-induced changes in the nucleus ambiguus and dorsal motor nucleus of the vagus (which contain the cell bodies of the efferent vagal neurons) suggest that SP binding sites on these neurons are lost with chromatolytic changes subsequent to axonal transection. The amount of SP binding in the NTS (which receives innervation from the afferent vagus) was not detectably altered by either VAG or removal of the afferent neuronal cell bodies in the nodose ganglion (NGX). This finding suggests that SP binding in the NTS is not regulated by vagal afferent innervation. The kinetics of binding may however be altered in a manner which is not detected by autoradiography. Supported by NIH grant HL-26849.

NEUROMODULATORS

350.1 DETECTION OF ADENOSINE A_1 RECEPTORS IN A CLONAL CELL LINE. L. R. Toll. Division of Life Sciences, SRI International, Menlo Park, CA 94025.

Adenosine and analogs have been found to interact with two independent membrane bound receptors with opposite biochemical consequences. Micromolar concentrations of adenosine stimulate A_2 receptors which are found in the brain and many peripheral tissues. Stimulation of A_2 receptors activates adenylate cyclase. Nanomolar concentrations of adenosine stimulate A_1 receptors which produces an inhibition of adenylate cyclase activity. Binding to A_1 receptors, which are thought to mediate the neuronal depressant activity of adenosine, has been demonstrated only in brain, testes and fat cells. I now report the presence of adenosine A_1 receptors in a clonal neuroblastoma cell line, N_2A. N_2A cells have previously been shown to possess receptors for GABA and benzodiazepines.

Binding studies have been conducted on membranes prepared from N_2A cells. Binding characteristics appear very similar to those of A_1 receptors found in brain, testes and fat cells. Binding was conducted with the high affinity, stable adenosine analog [^{3}H]cyclohexyladenosine([^{3}H]CHA). As in other tissues, the production of endogenous adenosine during the course of the incubation appears to inhibit [^{3}H]CHA binding. Consequently, binding is done in the presence of 0.2 units/ml adenosine deaminase to prevent the accumulation of adenosine.

Binding parameters in N_2A cell membranes appear similar to those in brain membranes. [^{3}H]CHA association is slow, requiring two hr to reach equilibrium at 25°C. Saturation isotherms reveal a single binding site with a K_D of 0.7 nM and a B_{max} of 90 fmol/mg protein. The Hill slope is 1.0, indicating no cooperativity of binding. Inhibition of [^{3}H]CHA binding by various adenosine receptor agonists displays the anticipated pharmacology and stereospecificity. 2-chloroadenosine and ℓ-phenylisopropyladenosine (ℓ-PIA) have nanomolar affinities, with d-PIA over 40 times less potent than the ℓ-enantiomer. The N_2A adenosine A_1 receptor is also regulated in a similar way by guanine nucleotides. [^{3}H]CHA binding is greatly decreased by 0.1 mM GTP, but not ATP. Presumably this indicates a similar coupling to adenylate cyclase as found in brain and fat cells. Studies are continuing to determine the suitability of N_2A cells as a model system to study adenosine A_1 receptors.

350.2 AUTORADIOGRAPHIC EVIDENCE FOR UPTAKE, CONCENTRATION AND AXONAL TRANSPORT OF INTRAVENOUSLY-ADMINISTERED ^{125}I-TRIIODOTHYRONINE IN DISCRETE NEURAL SYSTEMS IN RAT BRAIN. M. B. Dratman, F. L. Crutchfield*, J. T. Gordon*, M. Murray*, M. E. Goldberger*, T. O. Allen*, and N. T. Adler*. Medical Research Service, VA Medical Center; Depts. of Medicine and Anatomy, Med. Coll. of Pa., and Dept. of Psychol., Univ. of Pa., Philadelphia, Pa. 19104.

Functional, biochemical, and autoradiographic observations have indicated that thyroid hormones are the source of neuroactive iodocompounds in rat brain. After i.v. administration, ^{125}I-labeled hormone is concentrated within selected cellular assemblies, short processes, and synapses in cerebral and spinal cord grey matter. To determine whether iodothyronines eventually appear in white matter, thaw-mount autoradiograms (ARGs) were prepared from serial sections of rat brains obtained 1,3,10,24 and 48 hours after i.v. ^{125}I-triiodothyronine (T_3^*). Brain sections were exposed to high-sensitivity film for 10-20 days; the developed ARGs revealed that T_3^* distribution patterns were highly modified with time after hormone administration. Observations through 10 hours confirmed previous evidence of concentration within selected regions of grey. However, by 24 and 48 hours, well-defined shifts in the labeling patterns indicated movement of iodocompounds from one brain region to another through the mechanism of axonal transport. Thus: early extensive labeling of cerebral cortex diminished with time and became laminated in appearance, concomitant with appearance of label in the corticospinal tract; early strong labeling of dentate gyrus was later associated with the appearance of well-defined labeling in the fornix; heavy accumulation of silver grains in inferior colliculus diminished while labeling in the medial geniculate body appeared and became prominent. Biochemical studies performed to determine the nature of the iodocompounds found in brain showed that although a number of metabolites had formed, iodide accounted for less than 7% of the total radioactivity while more than 70% migrated (paper chromatography) or co-eluted (HPLC) with T_3. Because axonal transport is an essential process for maintaining the functional integrity of the entire neuron, evidence that iodocompounds are transported by this mechanism provides further support for their participation in important neural functions; selectivity of localization conforms with their proposed role as precursor amino acids in protein and neurotransmitter pathways of metabolism. Supported by funds from VA Medical Research Service and NSF Grant #BNS 8210354.

350.3 CHARACTERIZATION, EXTRACTION AND FRACTIONATION OF GTP-PREFERRING PROTEIN KINASE ACTIVITY FROM NEURAL MEMBRANES. Y.H. Ehrlich, R.S. McCollum*, T.B. Davis*, W.C. Ohlsson*, J. Ellis* and R.H. Lenox. Neuroscience Research Unit, Dept. of Psychiatry and Dept. of Biochemistry, Univ. of Vermont Coll. Med., Burlington, VT 05405.

GTP plays a crucial role in the regulation of many cellular functions. In particular, several receptor-associated activities are known to be regulated by GTP. In some functions, unhydrolyzable analogs such as GppNHp can substitute for GTP in the regulation, indicating that binding to a regulatory component is the underlying mechanism. However, certain activities such as inhibition of adenylate cyclase by alpha$_2$-adrenergic receptors and agonist-induced desensitization of hormone-coupled adenylate cyclase in cell free preparations were shown to require the presence of native GTP. One mechanism that may be involved in such regulation is a process in which protein kinase utilizes GTP as a phosphate donor in the phosphorylation of specific membrane-bound proteins. We have reported (Biochem. Biophys. Res. Comm. 107:699-706, 1982) that preparations containing synaptic membranes from rat brain contain endogenous kinase activity which prefers to utilize GTP over ATP in the phosphorylation of specific protein components with apparent M.W. of 54,000 daltons (54K) and 33K. Characterization of this endogenous activity revealed that the preference for GTP requires the presence of Mn++ ions in the reaction medium. However, as little as 50 µM $MnCl_2$ is sufficient to support this activity in a reaction containing 1 mg/ml membrane protein and 1 µM gamma-^{32}P-GTP. Extraction of the membranes with 1.5% Triton-X-100 solubilized endogenous activity which phosphorylated the 54K protein with GTP. Removal of the Triton with Bio-Beads SM2 revealed preferential phosphorylation by GTP of another protein substrate with apparent M.W. of about 68K. This activity could be separated from ATP-utilizing phosphorylation systems by gel filtration on Sepharose-4B. In a separate investigation, we have detected Mn++-dependent phosphorylation by GTP of a protein migrating in SDS gel with M.W. of about 54K in membrane preparations from the neuroblastoma x glioma hybrid cells NG108-15 grown in culture. Preliminary experiments indicate that this activity increases when the cells are induced to differentiate by treatment for 4 days with di-butyryl cAMP. Homogenous population of these cells may represent an ideal model system for studying the role of GTP-supported protein phosphorylation in neuronal function. Supported by USPHS grant MH35735 and NSF grant BSN82-09265.

350.4 ENDOGENOUS ESTROGENS REGULATE DOPAMINE AUTORECEPTOR SENSITIVITY. G.U.Corsini*, M.P.Piccardi* and F.Bernardi* (SPON: G.Di Chiara). Clinical Pharmacology, University of Cagliari, Via Porcell 4, Cagliari, Italy.

Recent data from our laboratory indicated that estrogens, exogenously administered, induce a hyposensitivity of dopamine (DA) autoreceptors in male and in ovariectomized female rats (OVX) as well. Male rats (23 animals), when challenged with a low dose of apomorphine (20 ug/kg subcutaneously), show a marked decrease of motility (residual motility x = 29%) in almost all the animals (S.D. = 12.98). Similarly OVX rats (27) show a homogenous and substantial reduction of motility (x = 30%, S.D. = 11.14). On the contrary, female intact rats (21) show a moderate hypomotility (x = 49%) with a wide interindividual variation (S.D. = 17.33). This variation in female rats has been evaluated in relation to endogenous 17 B Estradiol (E) plasma levels in each animal. A positive correlation between E plasma levels and motility after apomorphine (r = +0.66) has been obtained with a statistical significativity (p 0.05). Ovariectomy (5, 10 and 15 days after) reduces the variability and potentiates the behavioural response to apomorphine in a way which is closely related to estrogen decrease. Infact 5 days after OVX, E plasma levels are 61.60 ± 18.23 pg/ml and the residual motility after apomorphine is 60% (sham operated animals show E plasma levels of 64.51 ± 21.12 pg/ml and residual motility of 65%). 10 days after OVX, E is 46.77 ± 4.00 pg/ml and motility is 54% and 15 days after OVX, E is 17.47 ± 4.02 pg/ml and motility is 27%. These results indicate that estrogens regulate physiologically DA autoreceptor sensitivity. These data may account for sex differences in the pharmacological manipulation of dopaminergic transmission.

350.5 NEUROTRANSMITTERS MODULATE IN VIVO PGE_2 RELEASE INTO CAT VENTRICULOCISTERNAL PERFUSATES. Silvia Divinetz Romero*, Jia-Yi Wang* and Tony L. Yaksh* (SPON: D.W. Klass). Dept. of Neurosurgery, Mayo Foundation, Rochester, MN 55905.

A great deal of work has accumulated indicating that prostaglandins (PGs) exert a negative feedback effect on neurotransmitter release at the sympathetic synapse. On the other hand, evidence supporting the role of neurotransmitters in regulating PGs synthesis is elusive. This study examines the effect of several neurotransmitters on the release of PGE_2 (the main cyclo-oxygenase product in the cat) in a preparation known to respond with increased PGE_2 levels when stimulated with depolarizing agents. Consecutive 30-60 min samples were collected from ventriculocisternal perfusions (100-200 µl/min) in chloralose-urethanized cats, before, during and after the addition of carbachol (CBC), norepinephrine (NE), dopamine (DA) or γ-aminobutyric acid (GABA). Samples were submitted to acid organic extraction and radioimmunoassay (RIA) with a specific antibody (sensitivity 14 pg; lower limit of detection 4 pg; cross reactivity: TxB_2; 6-keto $F1\alpha$; $F2\alpha$; PGD_2 <0.1%). Displacement curves of ligand binding with control and stimulated samples were parallel to those observed with PGE_2. In addition, the activity in perfusates was shown to co-migrate in a 2-solvent TLC system with PGE_2 standards, suggesting that the immunoreactive-PGE_2 cannot be distinguished from authentic PGE_2.

PERFUSED AGENT	N	PGE_2 RELEASE (pg/min); MEAN ± SE		
		Basal	Stimulated	Δ
CBC (10^{-4}M)	5	600±259	1,582±577*	+982±375
NE (10^{-4}M)	5	459±100	621±110*	+162± 58
DA (10^{-4} to 10^{-3}M)	5	603±157	293± 97*	-310± 96
GABA (10^{-4} to 10^{-3}M)	3	866±215	428±160*	-438±133

* $p < 0.05$

These data show: 1) That CBC and NE exert a stimulatory effect on PGE_2 synthesis. This effect of CBC, in preliminary experiments, is antagonized by pretreatment with atropine. 2) DA and GABA were shown to reliably reduce the control levels of PGE_2 in ventriculocisternal perfusates. These results suggest that the effect of centrally activating selected receptor populations will alter the resting levels of PGE_2 in the extracellular space. This regulation of PGE_2 release by putative neurotransmitter systems opens the possibility of an intricate inter-relationship between neurotransmitter release and eicosanoid bioregulators originating from membrane phospholipid pools. Distinct changes in eicosanoids would alter in turn the release and effects of other transmitters as well as the neuronal metabolic environment through local modifications in the microcirculation of particular areas of the brain. (Mayo Foundation and NSO6663)

350.6 PROSTAGLANDIN E_1 REDUCES BOTH THE DURATION OF AFTERPOTENTIALS AND THE AMPLITUDE OF THE CA^{++}-DEPENDENT ACTION POTENTIALS IN SYMPATHETIC NEURONS. D. Higgins, M. Klein* and H. Burton. Dept. Anatomy, Washington Univ. Med. Sch., St. Louis, MO 63110.

Prostaglandins of the E class inhibit the release of norepinephrine from axons of sympathetic nerves in vivo. To understand the mechanisms by which this autocoid alters transmitter release, intracellular recordings were obtained from the somata of perinatal rat sympathetic neurons maintained in tissue culture. Prostaglandin E_1 (PGE_1) (25-1000 ng/ml) did not cause a detectable change in either the resting membrane potential or the input resistance. However, PGE_1 altered the active responses of sympathetic neurons. Action potentials in these cells are followed by long (200-500 msec) hyperpolarizing afterpotentials. In the presence of PGE_1, the duration of the afterpotential was substantially (often >50%) reduced; such effects were not observed with PGA_2 or $PGF_{2\alpha}$ (1 µg/ml). Since the K^+ conductance responsible for the long afterpotential is Ca^{++}-dependent in these neurons, we tested the effects of PGE_1 on Ca^{++}-dependent action potentials. Action potentials recorded in the presence of tetrodotoxin (0.6 µM) and tetraethylammonium (5 mM) were reduced in amplitude by inorganic Ca^{++} antagonists (Co^{++}, Cd^{++}) and their amplitude varied with changes in the concentration of Ca^{++}. In most cells, PGE_1 (25-1000 ng/ml) either reduced or eliminated such Ca^{++}-dependent action potentials. Half maximal responses were observed with concentrations between 100 and 300 ng/ml; smaller effects were observed with concentrations of 25 to 40 ng/ml. We conclude that prostaglandins of the E class may act as neuromodulators by affecting Ca^{++}-dependent processes in sympathetic neurons. (Supported by NIH Grants: NS 14416, NS 09809, NS 07071 and RR 05389.)

350.7 A MODULATORY OCTOPAMINERGIC NEURONE INCREASES CYCLIC AMP LEVELS IN LOCUST SKELETAL MUSCLE. Peter D. Evans. (SPON: J.S.Kelly). ARC Unit of Insect Neurophysiology and Pharmacology, Dept. of Zoology, University of Cambridge, Downing Street, Cambridge, CB2 3EJ. U.K.

Stimulation of the octopaminergic neurone (DUMETi) to the extensor-tibiae muscle of the hindleg of the locust (Schistocerca americana gregaria) increases the levels of cyclic AMP, but not cyclic GMP, in the muscle in a frequency dependent manner. The response is blocked by phentolamine, an α-adrenergic blocking agent that also blocks the effects of octopamine in this preparation (Evans, 1981, J. Physiol., 318, 99). Octopamine increases cyclic AMP levels, but not cyclic GMP levels, in this muscle in a dose-dependent way. The response peaks after a 10 min exposure and then declines to a plateau. The effects are potentiated in the presence of the phosphodiesterase inhibitor, IBMX. The response is stereospecific for the naturally occurring D(-) isomer of octopamine and is also specific for monophenolic biogenic amines. Studies with a range of synthetic agonists and antagonists reveal that the receptors mediating the response are of the $OCTOPAMINE_2$ class.

$OCTOPAMINE_2$ class receptors occur both presynaptically on the terminals of the slow motorneurone and postsynaptically at extrajunctional sites on the muscle surface. The presynaptic receptors potentiate both the neural and spontaneous release of transmitter whilst the postsynaptic receptors increase the rate of relaxation of tension generated by the fast and the slow motorneurones in this muscle. Elevation of cyclic AMP levels using IBMX, forskolin (the diterpene activator of adenylate cyclase) and 8(4chlorophenylthio) cyclic AMP mimic the physiological effects of stimulating both the pre- and post-synaptic $OCTOPAMINE_2$ receptors.

The magnitude of the octopamine-mediated increase in cyclic AMP level varies in different regions of the muscle. It is highest in the regions that contain the largest number of slow and intermediate fibres. Thus in other muscles of the locust (and in muscles of other insects) which have different proportions of fast, intermediate, and slow fibres from that of the extensor-tibiae muscle of the hindleg, and where pre- and post-synaptic mechanisms may contribute to differing extents, octopamine will produce a different effect on tension in the muscle.

350.8 CENTRAL EFFECT OF PHOSPHOLIPASE A_2 ON TEMPERATURE IN CONSCIOUS RABBITS. S.B. Kandasamy* and B.A. Williams* (SPON: S.D. Corbin). Biosystems Division, NASA-Ames Research Center, Moffett Field, CA 94035.

Arachidonic acid and its metabolites are implicated in fever. Arachidonic acid is believed to be released by the activation of phospholipase A brought about by various types of stresses. The present study was undertaken to determine the effect on temperature of phospholipase A_2 in rabbits and to find out the effect of a cyclooxygenase inhibitor, indomethacin, an α-adrenoceptor antagonist, phenoxybenzamine, a prostaglandin antagonist, SC-19220 and a steroidal and non-steroidal blocker of phospholipase A activation, dexamethasone and mepacrine respectively on phospholipase A_2 -induced temperature response. Intracerebroventricular (ICV) administration of 1-10 units of phospholipase A_2 induced a dose-related hyperthermia. The hyperthermia induced by a submaximal dose of phospholipase A_2 (3 units) was tested with following drugs. Indomethacin (1-5 mg/kg, SC), phenoxybenzamine (0.3-1 mg/kg, IV) and SC-19220 (10-50 µg, ICV) attenuated phospholipase A_2-induced hyperthermia. 10-300 µg, ICV of dexamethasone and mepacrine did not antagonize phospholipase A_2-induced hyperthermia. These results indicate that phospholipase A_2-induced hyperthermia may be mediated via arachidonic acid, prostaglandins and norepinephrine.

350.9 NEUROHORMONAL MODULATION OF CRUSTACEAN VENTILATORY AND CARDIAC RHYTHMS. J.L. Wilkens, A.J. Mercier, R. Aramant* & T.M. Leah*, Dept. of Biology, Univ. of Calgary, Calgary, Ananda T2N 1N4

In many crustaceans forced activity, surgery and other 'stressful' stimuli cause prolonged (up to 24 h) changes in ventilatory (f_R) and to lesser extents heart (f_H) rates. Short duration (up to 20 min) startle and spontaneous pauses also occur in both systems followed by recovery periods of similar short durations. The short duration responses are probably neurally mediated whereas the long duration responses may be hormonally mediated. We have attempted to study the long duration responses in Carcinus maenas by i) assaying the hemolymph of stressed crabs for active substances, ii) administering known hormonal substances in vivo, and iii) testing the in vitro effects of neurohormones on the ventilatory pattern generators of the crab thoracic ganglion.

i) Crabs receiving hemolymph from stressed donors (10 min air exposure and sham surgery) exhibited cardiac arrest and apnea for 5 min followed by depressed f_R over the next 2 h. Crabs receiving saline or unstressed donor hemolymph exhibited a 30-40 min elevated f_R and slight tachycardia.

ii) The responses of crabs to injections of neurohormones are as follows: pericardial organ (PO) extracts induced slight tachycardia (N.S.) and increased f_R (32%): these responses lasted 2 h after single injections. 5-HT and octopamine (oct) produced similar responses to PO extracts. Proctolin increased f_R and f_H at low hemolymph conc.'s (10^{-10}M), but was inhibitory at higher levels (10^{-8}M). Dopamine (dop) produced shorter duration stimulation of f_R and f_H with a return to control rates in 1h. Vasotocin, x-organ and nerve extracts were without effect.

iii) Neurohormones were tested on in vitro perfused thoracic ganglia which continued to generate ventilatory neural patterns for up to 8 h. These ganglia exhibited high thresholds for 5-HT (10^{-3}M), oct (10^{-4}M), dop (10^{-3}M) and proctolin (10^{-5}M). There was considerable variability between repeated trials on any single ganglion and between ganglia. These ganglia were unresponsive to dopa, nor-epinephrine, substance P, somatostatin and leu-enkephaline.

There are similar trends in the responses of all 3 groups. The biogenic amines known to be present in the PO's and their anterior ramifications stimulate f_R and f_H whenever they are effective. The high thresholds for responses in group iii crabs are unexplained, but may indicate extraganglionic receptor sites for active agents. The inhibitory effects of stressed donor hemolymph are similar to some aspects of 10^{-8}M proctolin injections. It is concluded that long duration ventilatory and cardiac responses to 'stress' have a neurohormonal basis.

350.10 IS 2-AMINOETHANOL RELATED TO SYNAPTIC ACTIVITY IN THE RAT CORTICO-PONTINE PATHWAY? H. Perschak*, M. Wolfensberger*, M. Cuénod (SPON: D.W. Sirkin). Brain Res. Inst., University of Zurich, Switzerland.

Free 2-aminoethanol (ethanolamine, EA) is known to be incorporated into membrane phospholipids as exchange for another base. Such enzyme-catalyzed base exchange reactions have been suggested to play a role in neuronal receptor function (Arienti and Porcellati, in: Receptors for Neurotransmitters and Peptide Hormones, Pepeu et al. eds., 1980, pp. 43-49). The extracellular concentration of EA has been shown to be more than doubled in the pigeon optic tectum upon optic nerve stimulation. When microiontophoretically applied to tectal neurons, EA enhanced the effect of glutamate or GABA, while having no action on its own (Wolfensberger et al., Neurosci. Lett. 32, 1982, 53-58). We report here the stimulation-dependent increase of free EA in another system, this time in a mammal.

In the pentobarbital-anestetized rat, the tip of a push-pull cannula was placed stereotaxically in the ventral part of the basilar pontine gray, which was continuously perfused with a bicarbonate-buffered Ringer solution. The cortico-pontine fibers were stimulated by means of a bipolar electrode (a total of 40 impulses/sec. in trains of various patterns, four times threshold) at the level of the ipsilateral cerebral peduncle immediately rostral to the substantia nigra. The EA content of fractions collected in four-minute periods of rest or stimulation was determined by glass capillary gas chromatography with nitrogen-sensitive detection or selected ion monitoring. EA elution was measured at 9.8 ± 6.3 pmol/min under resting conditions and increased by a factor of 2.2 upon stimulation of cortico-pontine fibers.

These results suggest that extracellular free EA is related to synaptic activity.

Supported by the Swiss National Science Foundation grants 3.228.82 and 3.506.79 and the Dr. Eric Slack-Gyr-Foundation.

350.11 CHARACTERIZATION OF BINDING OF NERVE GROWTH FACTOR TO α_2-MACROGLOBULIN. P.H. Koo*, T.J. Teyler**, AND R.W. Stach***. *PROGRAM IN MICROBIOLOGY AND IMMUNOLOGY, **PROGRAM IN NEUROBIOLOGY, NORTHEASTERN OHIO UNIVS. COLLEGE OF MEDICINE, ROOTSTOWN, OH 44272. *** DEPARTMENT OF BIOCHEMISTRY, STATE UNIVERSITY OF NEW YORK UPSTATE MEDICAL SCHOOL, SYRACUSE, NY 13210.

The 2.5S nerve growth factor (βNGF) has been reported to bind to α_2-macroglobulin (α_2M) in serum (Ronne, H., et al. Biochem. Biophys. Res. Comm., 87: 330, 1979). Recently we have studied the kinetics, stochiometry and mechanism of this binding reaction. Radiolabeled βNGF injected i.v. was found associated with the mouse α-macroglobulin in plasma (αM, a homologue of human α_2M; Hudson, N.W. and P.H. Koo, Biochem. Biophys. Acta. 704: 290, 1982), and the binding is sufficiently tight to resist separation during gel filtration, polyacrylamide gel electrophoresis and immunoprecipitation of αM. The extent of binding of βNGF to αM is dependent upon the concentrations of the interacting components. As determined by double-antibody radioimmunoassays and Scatchard analysis, up to about 0.76 ± 0.2 mole of βNGF is found bound to each mole of αM, with an apparent association constant of $(2.0 \pm 0.8) \times 10^7\ M^{-1}$.

βNGF can be readily dissociated from αM in SDS gel electrophoresis in the absence of a reductant. Procedures which affect the proteinase-binding activity of αM (such as modification of αM with methylamine, or saturation of αM with anhydrotrypsin in the presence of soybean trypsin inhibitor) do not affect the binding of βNGF to αM. Hence βNGF is noncovalently associated with αM at a site separate from that of the proteinase binding sites of αM. Although sensitive to radioiodination procedures, the binding activity of βNGF to αM is rather resistant to tyrosine modification by tetranitromethane and heat (56°, 45 min.), but is labile to trypsin digestion. Since α_2M is a major serum proteinase inhibitor and most of the circulating βNGF is associated with α_2M, the possible role of α_2M as a modulator of βNGF activity is being investigated. (P.H. Koo is partially supported by grants from Pediatrics of Akron, Inc., OH; United Way Health Foundation of Stark County, OH; and NCI (#CA24337). R.W. Stach is supported by NIH Grant NS12325).

350.12 pH EFFECTS ON CALMODULIN PARTITIONING IN MOLLUSCAN NERVOUS SYSTEM AND RAT STRIATUM. Marlene A. Wilson* and Rhanor Gillette. (SPON: E. J. Roy). Neural and Behavioral Biology Program and Dept. of Physiology and Biophysics, Univ. of Illinois, Urbana, IL 61801.

Neuromodulatory pathways mediating the effects of food stimuli induce prolonged burst episodes in feeding command neurons of the predatory snail Pleurobranchaea (M. Gillette, J.Neurosci., in press). Cyclic AMP also induces burst episodes through potentiating a slow inward current (Green and Gillette, this volume). Calmodulin antagonists and slight intracellular alkalinization (0.1 pH unit) mimic the effect of cAMP in potentiating inward current and inducing bursting (Green and Gillette, Brain Res., in press; Gillette, J.Neurophysiol., 49: 509). Evidence was found for a calcium-calmodulin activated cAMP phosphodiesterase (PDE) in the nervous system which could mediate the effects of pH and calmodulin antagonists (Calhoon and Gillette, Brain Res., in press). These results led us to examine the pH dependence of calmodulin distribution, which could in principle explain the pH sensitivity of PDE and cell activity.

We examined the effects of pH on calmodulin partitioning between the cytoplasmic and particulate fractions of Pleurobranchaea ganglia. Using radioimmunoassay, calmodulin content was measured in the pellet and supernatant fractions of crude homogenates adjusted to pH 7.0, 7.4, 7.6, and 8.0. Fractions were isolated by centrifugation at 105,000 x g for 1 hour following pH adjustment. The percentage of total calmodulin in the soluble fraction of homogenates increased between pH 7.0 and pH 8.0. Analysis of variance revealed a significant effect of pH on calmodulin distribution ($p < 0.009$, N=5 experiments). Mean percentages of soluble calmodulin were 63.4 ± 4.5, 67.8 ± 3.7, 74.3 ± 1.3, and 80.2 ± 2.2 for pH 7.0, 7.4, 7.6, and 8.0, respectively.

With calcium electrodes we measured calcium concentrations in the homogenates to be between 25 and 200 μM. In order to evaluate pH dependence of calmodulin partitioning at $[Ca^{++}]$ nearly resembling intracellular levels, we titrated $[Ca^{++}]$ in the various aliquots to 1.6 μM with EGTA using a calcium electrode. Under these conditions, the pH/partitioning function increased steeply between pH 7.0 (49% soluble) and pH 7.4 (83% soluble). This pH range is typical of intracellular values.

We also investigated the effects of pH on calmodulin distribution in homogenates of the rat striatum. The pH/partitioning curve was V-shaped between 7.0 and 8.0. The percentage of total calmodulin in the soluble fraction was 71% at pH 7.0 and 8.0, but decreased to 59% at pH 7.4. These effects of pH were statistically significant ($p=0.01$, N=2 experiments).

(Supported by grants NSF BNS 79-18329 to RG and PHS-5-T32 GM07143 to MAW.)

350.13 IN VIVO ELECTROCHEMICAL AND UNIT RECORDING IN THE STRIATUM OF THE RAT FOLLOWING INFUSION OF AMPHETAMINE. R. L. Wilson*, K. Kamata*, K. D. Alloway, G. V. Rebec, and R. M. Wightman. Departments of Psychology and Chemistry, Indiana University, Bloomington, IN 47405

Intraperitoneal or intravenous administration of D-AMPH increases the concentration of ascorbic acid (AA) in neostriatal extracellular fluid of the immobilized or anesthetized rat as determined by in vivo electrochemistry. In contrast, the change in unit activity response in the striatum is dependent on the dose of amphetamine: unit activity decreases with lower doses (<2.5 mg/kg) and increases with higher doses (7.5 mg/kg). The unit activity changes are thought to reflect an increased level of synaptic dopamine that is induced by amphetamine. However, considerable evidence exists which suggests that AA can modulate the activity of dopamine receptors (Ewing et al., Brain Research, 261:101, 1983). Therefore, local application of D-AMPH in various regions of the brain has been examined to determine whether 1) AA can be increased in the extracellular fluid of the striatum by local application, and 2) to determine the effects of these local applications of D-AMPH on unit activity in the striatum. An increase in AA is not detected with local infusion of D-AMPH in the neostriatum. Unilateral infusion of D-AMPH into the substantia nigra, a major source of neostriatal afferent DA neurons, results in the bilateral increase of a substance which has the voltammetric characteristics of AA. This identification was accomplished through the use of two different types of working electrodes: disk shaped microvoltammetric carbon fiber electrodes and electrochemically modified electrodes (Gonon et al., Anal. Chem., 53:1386, 1981). In contrast, unit activity alters in an opposite fashion on each side of the brain: unit activity increased on the ipsilateral side, while unit activity on the contralateral side generally decreased. The unit activity changes correlate with the reported changes in DA concentration with D-AMPH infusion into the substantia nigra (decrease ipsilateral and increase contralateral) (Cheramy et al., Nature, 289:537, 1981). However, the unit activity responses also correlate positively with the increase of AA concentration on both sides with respect to experimental success and response duration.

350.14 CORTICOSTERONE AND LONG-TERM POTENTIATION IN THE HIPPOCAMPUS. A.V. Nowicky, R.M. Vardaris and T.J. Teyler. Neurobiol. Area Com., Dept. of Biol. Sci., Kent State Univ., Kent, O. 44242.

Effects of corticosterone (CT) on long-term potentiation (LTP) of Shaffer-collateral/CA1-pyramidal cell synapses was investigated using in vitro slice preparations of hippocampus from adrenalectomized (ADX) male rats. Changes in synaptic efficacy were monitored by measuring the mean amplitude and latency of extracellular population spikes elicited by afferent volleys set up in the Schaffer collaterals. The LTP protocol involved 1) a pre-LTP control series: an input-output (IO) function was obtained by incrementing stimulus voltage from threshold to maximum population spike amplitude; 10 stimuli producing a 1 mV amplitude were presented at 30-sec intervals; 2) LTP induction: the stimulus was delivered at 100Hz for 1 sec; 20 min later, 10 single stimuli were presented at 30-sec intervals; the pre-LTP IO function was repeated with the original stimuli. This LTP protocol was performed before and 20 min after administration of 0, 4, or 7nM CT by addition to the bathing solution. The lower CT concentration corresponds to a normal morning level in hippocampus, and the 7nM dose reflects the evening level.

Field potential amplitudes were enhanced after LTP induction in most slices. The population response amplitudes tended to be increased relative to the steroid-free control group after administration of CT. These results indicate that LTP can occur in sub-field CA1 of hippocampus from ADX rats and that restoration of normal levels of CT can enhance this effect. Prior work has shown that the incidence and amount of LTP in hippocampal slice preparations is correlated with the diurnal light cycle, such that CA1 pyramidal cells show more LTP when obtained during the light period and granule cells of area dentata show more in the dark period (Harris, K.M. and T.J. Teyler, Brain Res., 261:69-73, 1983). Synaptic throughput in CA1 of hippocampal slice preparations was enhanced by 4nM CT (morning level) more than by 7nM CT (evening level) (Reiheld, C.T., et. al. Neurosci. Abst., 8:970, 1982). In addition it has been found that LTP in the dentate gyrus of intact rats is abolished by adrenalectomy, but can be restored by injections of CT (Dana, R.C., et. al., Neurosci. Abst., 8:316, 1982). It appears that CT may modulate the magnitude of LTP in the hippocampal formation. This modulation may be the mechanism underlying circadian rhythms for LTP. The present findings suggest that CA1 of the hippocampus may be less dependant on normal levels of CT than dentate gyrus with respect to induction of LTP.

(Supported in part by NINCDS Research Grant 16507).

350.15 ADENOSINE REDUCES DEPOLARIZING RESPONSES TO LOCALLY APPLIED GLUTAMATE IN PYRAMIDAL NEURONS OF RAT HIPPOCAMPUS IN VITRO. W.R. Proctor and T.V. Dunwiddie, Dept. of Pharmacology, Univ. Col. Health Sci. Ctr., and VA Medical Center, Denver, CO 80262.

Adenosine has a well-established depressant action on excitatory post-synaptic potentials in the CA1 region of rat hippocampus. Extracellular potentials, such as the field EPSP and population spike, are markedly reduced in hippocampal brain slices by perfusion with low (5-10 uM) concentrations of adenosine. Intracellular recordings from pyramidal cells show a parallel decrease in the amplitude of the EPSP elicited by *Schaffer collateral and commissural fiber stimulation*. Although it generally has been assumed that this depression by adenosine occurs at the pre-synaptic level (i.e., via a reduction in the amount of transmitter release), we have recently demonstrated direct post-synaptic effects of adenosine (e.g., a marked increase in calcium spike threshold). Thus at low concentrations of adenosine, there is a significant effect on the post-synaptic membrane that cannot be accounted for by pre-synaptic actions.

We now show further evidence for a post-synaptic interaction of adenosine with locally applied glutamate. In our paradigm, intracellularly recorded pyramidal neurons were stimulated alternately with 1) a synaptic input, 2) hyperpolarizing current pulses, 3) local application of glutamate (100 msec duration pressure ejection from glass micropipettes) in the cell body layer, and 4) local application of glutamate from a second pipette in the dendrites (stratum radiatum). Depolarizing responses to locally applied glutamate, either near the soma or on the apical dendrites, were significantly reduced by adenosine. However, the magnitude of the depression of synaptic potentials was typically about twice that seen in the depolarizing responses to glutamate; in 35/38 trials the synaptic response was depressed to a greater extent than was the response to glutamate. The concentration of adenosine required to depress the depolarizing response to glutamate by 50% (130 uM; 95% confidence limits = 92-170 uM) was significantly higher than that required to reduce the EPSP by the same extent (22 uM; 95% confidence limits = 7-76 uM).

If the post-synaptic response to the endogenous neurotransmitter is affected in the same fashion as are responses to locally applied glutamate, this data would suggest that there is a substantial post-synaptic component to the depressant effect of adenosine on synaptic transmission in the hippocampus.

This research was supported by grants VA 394463116-01 and DA 02702 to T.V.D., and grant DA 07043-07 to W.R.P.

350.16 INFLUENCE OF ADRENAL STEROIDS ON APOMORPHINE-STIMULATED CEREBRAL GLUCOSE UTILIZATION. R. H. Sundermann*, A. W. Toga and A. C. Jordan*. Depts. of Psychiatry and Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110

Glucocorticoids have great therapeutic value in a variety of autoimmune, hypersensitivity, and neoplastic conditions. However, their use can be complicated by the development of serious neuropsychiatric dysfunction including depression, mania, and psychoisis. As part of a study to elucidate possible mechanisms of steroid-induced mental status changes, we studied the effects both of adrenalectomy and of pharmacologic doses of glucocorticoid on apomophine-stimulated regional cerebral glucose utilization.

Male albino rats were divided into three groups: 1) control, 2) those undergoing bilateral adrenalectomy 2 weeks before sacrifice, and 3) those given daily injections of corticosterone (2mg/kg) for 2 weeks before sacrifice. Control and adrenalectomized rats were given daily injections of drug vehicle only. In all animals, apomorphine (1mg/kg) was given intravenously 2 minutes before the 14C-2-deoxyglucose (60 μ Ci/kg) was given. This dose of apomorphine resulted in marked stereotypic behavior over the ensuing 50 minutes, immediately prior to sacrifice.

Regional cerebral glucose utilization was calculated using the Sokoloff equation. Adrenalectomy had little or no effect on apomorphine-stimulated glucose utilization in the nucleus accumbens, olfactory cortex, and motor cortex. However, it did result in increased glucose utilization in the striatum, septum, substantia nigra, cingulate and primary sensory cortex by 27%, 31%, 36%, 24%, and 35% respectively. The treatment of otherwise intact animals with daily injections of corticosterone (2mg/kg/day) caused a small decrease (15-20%) in apomorphine-stimulated glucose utilization in the following regions: striatum, nucleus accumbens, septum, olfactory and motor cortex. A larger decrease (57%) was seen in cingulate cortex and a small increase (13%) in the substantia nigra.

As noted previously by McCulloch et al (Br. Res. 243:67-80, 1982), the distribution of alterations of energy metabolism produced by apomorphine is not singly correlated with the topography of dopamine receptors. Our results confirm this and additionally indicate that changes in adrenal steroid status have marked effects on specific regions of apomorphine-stimulated glucose utilization.

350.17 LONG-TERM-ENHANCEMENT (LTE) OF SLOW (S-)EPSP, INDUCED BY A PHYSIOLOGICAL PREGANGLIONIC CONDITIONING TRAIN, EITHER HOMO- OR HETEROSYNAPTICALLY. Sumiko Mochida* and B. Libet, Dept. of Physiol., Univ. of California, San Francisco, CA. 94143.

The muscarinic slow depolarizing response to ACh or methacholine, MCh (Libet&Tosaka,1970) and the equivalent s-EPSP (Ashe&Libet,1981), had been found to exhibit LTE after a brief exposure to dopamine, DA, especially in presence of a COMT-inhibitor. We now show that a similar LTE of s-EPSP test responses regularly follows a single orthodromic conditioning train in curarized superior cervical ganglia (SCG) of rabbit and rat, and even when conditioning in a separate input line that is heterosynaptic to the test line (in stellate ganglion of cat).

S-EPSP test responses (single preganglionic volley or brief train) at 35°C, are readily increased by 50 to 100%, for 1 to 4 hours, following a conditioning preganglionic train (of 10 pps for 2 min or 3 pps for 7 min). Prior addition of gallamine (25 μg/ml) blocks most or all of the s-IPSP and frees the s-EPSP of confusion by the overlapping variable s-IPSP component, although the LTE of s-EPSP is usually quite obvious even without gallamine. No COMT-inhibitor is needed to show this LTE. The entire, enhanced s-EPSP is abolished by the muscarinic antagonist QNB ($5X10^{-8}$M).

In cat stellate ganglion, a conditioning stimulus train (10 pps-2 min) on the sympathetic chain below T-4 induces an LTE of s-EPSP elicited heterosynaptically in preganglionic ramus T-4 or T-3 (as well as an LTE of homosynaptic s-EPSP tests at the chain).

In rabbit SCG, the specific DA antagonists (3 to 7 μM) d-butaclamol, spiroperidol, and flupenthixol strongly depress the LTE induced by a 5 pps-2 min conditioning train. Adrenergic antagonists, whether α (dihydroergotamine, 7 μM) or β (sotalol, 10 μM), were without effect.

These results demonstrate that LTE of the muscarinic s-EPSP can be easily induced by physiological neural inputs; that the induction can be heterosynaptic and is not due to presynaptic PTP; and that such LTE is probably mediated by intraganglionic release of a second transmitter, namely DA in at least the rabbit SCG. (Supported by USPHS grant NS-00884.)

350.18 ENDOGENOUS MODULATOR OF THE BRAIN MUSCARINIC RECEPTOR--PRELIMINARY CHARACTERIZATION. R. Diaz-Arrastia, T. Ashizawa*, S. H. Appel, Departments of Neurology and Biochemistry and Program of Neurosciences, Baylor College of Medicine, Houston, Texas 77030.

We have found endogenous molecules that modulate the binding of a radiolabelled muscarinic antagonist, ^{3}H-quinuclidinyl benzilate (^{3}H-QNB), to the brain muscarinic acetylcholine receptor (MAchR). The modulating activity was found in extracts of calf thymus. Preincubation of washed rat brain synaptosomes with the extract inhibited the binding of ^{3}H-QNB as measured by a filter assay. The activity on the extract is heat stable, extractable in 0.1 M acetic acid, precipitable in ether, and resistant to neutral protease, papain, trypsin, and acetylcholinesterase. It elutes in the included volume of Biogel P_2 column in two fractions, A and B. We have achieved approximately a thousandfold purification of the active molecules by boiling, acid extraction, and gel filtration chromatography.

Kinetic studies show that preincubation of synaptosomes with either fraction increases the K_D and decreases the βmax of the receptor for ^{3}H-QNB. Hill coefficients were approximately 1.0 for fraction B but greater than 1.0 for fraction A. The effect of both fractions appeared reversible by washing the synaptosomes after the preincubation. Simultaneous addition of the fractions and ^{3}H-QNB to the synaptosomes failed to inhibit the binding. At least a 60 minute preincubation at room temperature was necessary for maximum inhibition. ATP (1mM) potentiated and EDTA (1mM) prevented the inhibition by either fraction.

These results suggest that endogenous molecules alter the binding of antagonists to the brain MAchR. We are currently attempting to purify and characterize the active molecules, to determine the mechanism of modulation and to define a physiological role for these molecules. (We acknowledge support from the Hartford and Kleberg Foundations).

350.19 SPECIFIC BINDING OF ANAPHYLATOXIN C3a TO A HYPOTHALAMIC MEMBRANE FRACTION OF THE RAT. C.A. Williams, N. Schupf and L. von Mechow*. Division of Natural Sciences, SUNY-Purchase, Purchase, NY 10577, Dept. of Psychology, Manhattanville College, Purchase, NY 10577.

C3a is a pharmacologically active basic peptide (9000 da) cleaved from the third component (C3) in the serum complement cascade. We have proposed that the anaphylatoxins C3a and C5a produced at sites of immune complex deposition in the brain could mediate the neuropsychiatric disorders often observed in immune complex diseases such as systemic lupus erythematosus (lupus cerebritis). Immune complex-forming systems administered focally via indwelling cannulae to the rat perifornical hypothalamus modifies drinking (Williams, C. and Schupf, N., Science, 196:328, 1977) and eating (Schupf, N. and Williams, C.A., Soc. Neurosci. Abstr. 3:400, 1977). We have also demonstrated that C3a (0.1 nmole) at this site potentiates norepinephrine (NE)-induced eating and carbachol-induced drinking, but that it has no effect in unstimulated rats (Seeman, B. et al., Soc. Neurosci. Abstr. 4:414, 1978). This activity resembles that of dopamine (DA) at this site and the C3a effect is inhibited by both haloperidol and phentolamine (PHT). One hypothesis for C3a activity is that it acts via NE/PHT-binding receptor to release endogenous DA.

The specificity of C3a binding to a hypothalamic membrane fraction (HM) was studied by a microfuge sedimentation method (C3a and C5a preparations were obtained from Dr. Tony Hugli). ^{125}I-C3a (0.1-2.5 nM) was incubated 30 min at 37°C with HM (30 µg, proteins/ml), with and without 0.5 µM cold C3a. A K_d of 0.8 nM was determined. C3a binding at 0.5 nM was not inhibited by 0.5 µM haloperidol, propranolol or anaphylatoxin C5a; PHT, 0.5 µM, inhibited C3a-specific binding by 42%. These results are consistent with the DA release hypothesis, but also suggest that there is a specific C3a receptor in the rat brain. There are such receptors on peripheral mast cells and circulating basophils and these cells are both degranulated by C3a or C5a by separate and distinct receptors. An alternative hypothesis, that C3a directly stimulates a DA receptor, resulting in the psychopharmacological effects observed, is not supported by these experiments in that haloperidol failed to compete for C3a binding sites.

This is the first report of anaphylatoxin binding to brain tissues. (These studies were supported by grant NS 17168 from the U.S. Public Health Service.)

350.20 ENKEPHALIN MODULATION OF CATECHOLAMINE ACTION ON THE HEART. J. A. Ruth* and L. E. Eiden*, (SPON: R. Borke). School of Pharmacy, University of Colorado, Boulder, CO 80309 and Lab. of Cell Biology, NIMH, Bethesda, MD 20205.

Met- and leu-enkephalin (LE) cause a dose-dependent attenuation of norepinephrine (NE)-induced positive chronotropy in isolated spontaneously beating rat atria (Eiden and Ruth, Peptides 3:475, 1982). The mechanism of this action has been further investigated. NE at 10^{-5} M elicited an increase in atrial rate of 149 ± 9 beats per minute (bpm) above basal rate (353 bpm) which was decreased to 89 ± 8 bpm (-40%) in the presence of 10^{-8} M LE. The effect of LE was completely reversed upon addition of 10^{-7} M naloxone. In order to determine the cellular locus of LE modulation of positive chronotropy elicited by catecholamines (CAs) the effect of LE (10^{-8} M) on isoproterenol (ISO) and forskolin (FOR) positive chronotropy was investigated. ISO at 10^{-5} M caused an increase in beating rate of 168 ± 16 bpm which was reduced to 123 ± 12 bpm (-25%) in the presence of 10^{-8} M LE. FOR at 10^{-5} M caused an increase in beating rate of 103 ± 11 bpm which was not altered (120 ± 9 bpm) in the presence of 10^{-8} M LE, demonstrating that LE affects specifically CA-induced positive chronotropy.

Enkephalin peptides may exert their action in a number of neuronal systems by altering Ca^{2+} flux across the cell membrane (Bixby and Spitzer, Nature 301: 431, 1983). Therefore, the effect of altering extracellular calcium on LE modulation of CA positive chronotropy in isolated atria was examined. Lowering extracellular calcium from 2.5 to 0.5 mM reduced the positive chronotropic action of 10^{-5} M ISO from 168 to 40 ± 14 bpm. In the presence of 10^{-8} M LE (and lowered calcium) however, 10^{-5} M ISO elicited an increase in beating rate of 112 ± 9 bpm; more than 100% greater than in the absence of LE. LE alone did not cause a significant change in the basal beating rate.

LE may act to alter CA-dependent fluxes of calcium and other ions in isolated atria, attenuating CA-induced positive chronotropy at normal calcium concentrations and increasing CA-induced positive chronotropy under conditions of low extracellular calcium.

This work was supported by USPHS Grant NS 18752.

SUBCORTICAL ORGANIZATION

351.1 THALAMIC AND MIDBRAIN PROJECTIONS OF NUCLEUS RETICULARIS THALAMI. J. Hada*, M. Steriade and A. Parent (SPON: R. Boucher). Dept. Physiol. and Anat., Laval Univ., School of Med. Québec, Canada, G1K 7P4.

The incompleteness of information concerning the relative degree of axonal projections from different areas of nucleus reticularis (RE) to various thalamic nuclei and the incertitude concerning a RE projection to the midbrain core prompted this investigation in cat and squirrel monkey by using the retrograde transport of horseradish peroxidase (HRP) and double fluorescent tracers (fast blue-FB and nuclear yellow-NY).

The only thalamic injections that left RE nucleus virtually free of labeling were located in the anterior (AV-AM-AD) nuclear group. In those experiments, evidence for the effectiveness of the technique was provided by heavy labeling of the mamillary nuclei. All the other injections in lateral (VA-VL, LP, VB), intralaminar (CL-PC, CM-PF) and medial (MD, VM) thalamic nuclei resulted in retrograde labeling of RE neurons. Judging from the number of labeled cells, the major targets of RE nucleus are the anterior (CL-PC) and posterior (CM-PF) intralaminar nuclei. While the projections to intralaminar nuclei arise in the rostral pole and the ventral wing of RE nucleus, the neurons projecting to lateral nuclei are mainly localized in adjacent RE zones. In various experiments with double fluorescent tracers, the number of RE positive cells following CL-PC or CM-PF injections exceeded the number of RE cells labeled following injections in one of lateral nuclear groups (VA-VL, VB or LP) by factors from 2 to 5. An overwhelming number of RE cells were found to be selectively labeled by one of the fluorescent tracers; double-labeled RE neurons constituted a proportion of only 2 to 6% of the total number of positive cells.

The projection from RE to superior colliculus and midbrain reticular core was found in experiments using retrograde transport methods and it was corroborated by antidromic invasion of slow-conducting RE cells to midbrain reticular stimulation.

These results point to a preferential projection from RE toward intralaminar thalamic nuclei and emphasize the quite specific organization of projections from different RE neuronal aggregates to various thalamic nuclear groups. The interconnections between RE and lateral or intralaminar thalamic nuclei are of a paramount importance for the genesis of spindle rhythmicity during slow-wave sleep (see companion paper by Steriade et al.).

Supported by MRC Grants MT-3689 and MT-5781.

351.2 ABOLITION OF SPINDLING RHYTHMICITY IN THALAMOCORTICAL CELLS DISCONNECTED FROM THE RETICULARIS THALAMI NUCLEUS. M. Steriade, M. Deschênes* and L. Domich*. Lab. Neurophysiol., Dept. Physiol., Laval Univ., School of Med., Québec, Canada, G1K 7P4.

Studies in this laboratory have shown that thalamocortical neurons in lateral (VA-VL, VB, LP) and intralaminar (CL-PC) nuclei exhibit during natural slow-wave sleep (S) or anesthesia slow rhythmic (≈0.1-0.2 Hz) episodes of hyperpolarization during which they oscillate within the frequency range of spindle waves (≈7-14 Hz). Since the most conspicuous slow rhythmicity (≈0.1-0.2 Hz) of spike barrages was recently observed in reticularis thalami (RE) neurons, we checked if interconnections between RE and thalamocortical cells were essential for the slow and spindle rhythmicity of the latter.

In acutely prepared cats under ketamine anesthesia, two transections (a frontal one at plane ≈11.5-12.0, and a parasagittal one at plane ≈5-6) produced two different groups of nuclei. Medial to the transection, we explored VA-VL and CL-PC neurons deprived of RE input. Laterally, we explored VB cells in the presence of the adjacent intact RE. About 85% of physiologically identified relay neurons in VA-VL and CL-PC nuclei discharged single high-frequency bursts (that betray preceding inhibitory periods) with a striking periodicity of ≈1 Hz to 2 Hz in different neurons. Small amounts of a short-acting barbiturate diminished the occurrence of isolated bursts in those neurons, instead of inducing the normal rhythmic spike clusters within spindling frequencies. During periods of EEG desynchronization, the solitary bursts were abolished and replaced by single spikes, at low firing rates. Similar results (i.e. single bursts at ≈1-2 Hz, lacking both the slow and spindling rhythmicities) were obtained in cortically projecting VA-VL and CL-PC neurons during natural S sleep in chronically implanted cats with lesions in the rostrolateral parts of RE nucleus. In contrast with the single bursts in RE-deprived relay neurons, normal VB neurons discharged during S sleep or anesthesia with the classical rhythmic sequences of repetitive grouped spike bursts that were replaced on arousal by sustained single discharges with increased firing rates.

We conclude that interconnections between RE and thalamic relay neurons are essential for the genesis of normal thalamic spindling. The presence of burst discharges in RE-deprived relay neurons indicates the presence of long-lasting hyperpolarizing periods similar to those observed in normal conditions. This suggests that RE neurons control the temporal pattern of inhibition-rebound sequences in relay neurons, instead of producing them. This would imply that RE neurons exert their control on intranuclear inhibitory interneurons.

Supported by MRC Grants MT-3689 and MT-5877.

351.3 RETICULARIS THALAMI NEURONS EXHIBIT TONICALLY INCREASED RATES OF SPONTANEOUS FIRING AND ENHANCED SYNAPTIC EXCITABILITY DURING EEG-DESYNCHRONIZED BEHAVIORAL STATES. L. Domich*, M. Steriade, G. Oakson* and J. Hada* (SPON: L. Larochelle). Lab. Neurophysiol., Dept. Physiol., Laval Univ., School of Med., Québec, Canada, G1K 7P4.

According to the rather widely accepted belief that neurons of reticularis thalami nucleus (RE) exert inhibitory effects upon cortically projecting (relay) neurons in other thalamic nuclei, one would expect reciprocal changes between RE cells and hypothetically inhibited relay cells by changing the state of vigilance. Work in our laboratory has established that, during both wakefulness (W) and desynchronized sleep (D) compared to slow-wave sleep (SWS), physiologically identified thalamocortical relay cells in VA-VL and intralaminar CL-PC nuclei increase their rates of spontaneous firing as well as the probability of their antidromically and monosynaptically elicited discharges and decrease the duration of the long-lasting inhibitory phase. We have now investigated in chronically implanted, behaving cats the neuronal activities in the rostral pole and rostrolateral part of RE nucleus that mainly projects to CL-PC and VA-VL nuclei (see companion paper by Hada et al) to learn whether RE neurons show opposite changes in excitability compared to thalamocortical CL-PC and VA-VL neurons. Instead of reciprocity, we found parallelism.

In addition to their histological localization, RE neurons were identified by their monosynaptically evoked spike bursts to cortical stimulation and spontaneously occurring SWS-related spike barrages (much longer in duration and with lower intraburst frequencies than those of thalamocortical cells). During SWS, spike barrages appeared rhythmically, with a periodicity of 4 to 8 seconds in different RE neurons. From the very onset of arousal from SWS and during the continuing steady state of W, RE cells significantly increased their rates of spontaneous firing, changed the long bursts separated by silent periods into a tonic discharge pattern, showed an increased synaptic excitability and exhibited a sharpening in the subsequent inhibitory processes. Aspects similar to those in W were observed during D sleep.

The above results clearly conflict with the idea of an inhibition exerted by RE neurons upon thalamocortical neurons, both cellular classes being characterized by similar features. If RE neurons are GABA-ergic, as recently shown, the possibility remains that the presumed inhibitory actions of RE cells are preferentially exerted on inhibitory interneurons intrinsic to various thalamic nuclei. Alternatively, GABA-ergic RE cells may exert depolarizing actions upon dendrites of thalamocortical cells, similarly to the effect described in hippocampal pyramidal cells.

Supported by MRC grant MT-3689.

351.4 EFFERENT PATHWAYS FROM THE NUCLEUS INTERCOLLICULARIS IN THE RING DOVE. T. R. Akesson, N. deLanerolle, and M.-F. Cheng. Institute of Animal Behavior, Rutgers University, Newark, NJ 07102 and Section of Neurological Surgery, Yale University, New Haven, CT 06510.

The importance of the nucleus intercollicularis (ICo) as a midbrain structure regulating vocalization has been demonstrated in several avian species. In the female Ring Dove (Streptopelia resoria), lesioning inhibits and implantation of estradiol facilitates production of a courtship vocalization which is essential for reproductive success (Cohen, J. and M.-F. Cheng, Brain Res., 207:279, 1981). In this study we have used autoradiography to identify efferent projections of the ICo.

A 0.1-0.2 µL volume of ^{3}H leucine(∿50 mCi/µL) was injected into the ICo and control injections were made in the tectum and the nucleus mesencephalicus lateralis, pars dorsalis (MLd) to distinguish ICo projections from those of surrounding tissue. ICo efferents were traced to the central gray and, proceeding rostrally, this pathway passes below the dorsal thalamus, mainly via the stratum cellulare internum to the periventricular area and the anterior medial hypothalamus.

ICo efferents also course ventrally in the lateral midbrain including regions which are termed nucleus mesencephalicus lateralis, pars ventralis and formatio reticularis lateralis mesencephali in the atlas of Karten and Hodos (1967). In the rostral midbrain labeling is mainly distributed medial to the pretectal and subpretectal nuclei and passes diffusely in an area that extends laterally to the tractus occipitomesencephalicus, ansa lenticularis, and tractus quintofrontalis and may include the stratum cellulare externum. Labeling can be followed in the stratum cellulare internum, passing dorsally over the posterior hypothalamus and extending to the periventricular area.

In the rostral midbrain, a discrete concentration of grains could be traced from dorsolateral to the nucleus ectomamillaris to the nucleus ovoidalis. This pattern corresponds to part of the known auditory pathway and control injections in the MLd resulted in dense labeling of this pathway. Injections in the ICo usually involved some of the medial border of the MLd. Therefore transport to the nucleus ovoidalis may have been via the MLd and not the ICo. A possible interaction between auditory and vocalization systems is discussed.

In conclusion, our results demonstrate connections from the ICo to areas that have been shown to be hormone-sensitive and influence the expression of reproductive behaviors.

Supported by NSF grant BNS-8121495 and RSD award K02-MH-70897 to M.-F. Cheng.

351.5 A LIGHT AND ELECTRON MICROSCOPIC ANALYSIS OF NIGRAL-TECTAL RELATIONSHIPS IN THE CAT. M. Behan, C.-S. Lin & W.C. Hall, School of Veterinary Medicine, Madison, Wisconsin, & Duke University Medical Center, Durham, North Carolina.

Substantia nigra pars reticulata contains neurons which respond before saccadic eye movements (Hikosaka & Wurtz,'81, Progress in Oculomotor Res., p.145-152) and project to the deep layers of the superior colliculus (Graybiel,'78, Brain Res., 143:339-348). Our experiments have two objectives. First, to characterize the neurons in the deep tectal layers which project to the brainstem reticular formation and spinal cord by way of the predorsal bundle and second, to determine the anatomical relationships between these neurons and the termination of the nigrotectal tract.

To characterize the morphology and fine structure of the cells of origin of the predorsal bundle, horseradish peroxidase was injected into the predorsal bundle as it crosses the midline, beneath the oculomotor nucleus. Using this approach, both the somas and most of the dendrites of these tectal cells can be homogeneously filled with HRP. These retrogradely filled neurons are located primarily in the stratum griseum intermediale, and most of them are large (30-60 um soma diameter) and multipolar. The dendrites, which have a few spines, branch frequently to form dendritic fields that may exceed 2 mm^2. Both the somas and dendrites are densely covered with synaptic terminals which contain either round or pleomorphic vesicles.

Following injections of ^{3}H-proline into the substantia nigra pars reticulata, terminals are labeled in the stratum griseum intermediale. The majority of these terminals contain pleomorphic vesicles and make symmetric contacts. Over one-third contact either initial dendritic segments or large dendritic profiles. Experiments combining these methods are in progress to determine the relationships between the nigrotectal terminals and cells of origin of the predorsal bundle.

Supported by NIH grants EY04478 and EY04060 and NSF grant BNS 8109794.

351.6 INTERNUCLEAR CONNECTIONS IN THE THALAMUS OF THE MONKEY. S. Jacobson, D.L. Kasdon* and R.J. Lechan*. Departments of Anatomy and Cell Biology, Neurosurgery and Endocrinology, Tufts University School of Medicine, Boston, MA 02111.

Studies on the circuitry within the cerebral cortex have demonstrated convergence within many of the pathways. Previous studies on the thalamus have not shown any parallel intrinsic circuitry and in fact have only shown very limited interconnections. In the current study we have used immunocytochemical methods to determine if there is an extensive interthalamic circuitry with any concomitant convergence in the thalamus of the Macaca fascicularis.

We have examined the thalamic circuitry after direct injections of the retrograde tracers HRP or WGA into the dorsomedial nucleus or the medial pulvinar nucleus. In order to directly visualize the thalamus, under general anesthetic, the corpus callosum was aspirated caudally and the HRP (25% aqu. sol.) or WGA (2% aqu. sol.) was delivered from a 10µl Hamilton Syringe into the dorsomedial nucleus on one side and the medial pulvinar of the other side. The animals were perfuse-fixed four days postoperatively with 4% paraformaldehyde at pH 7.4 and sectioned at 60µ on a sliding microtome with a freezing attachment. The antisera to HRP and WGA were used to demonstrate the interthalamic circuitry.

The injection into the medial pulvinar nucleus produced labelled cells in the reticular, dorsal lateral geniculate and nucleus limitans while the injections into dorsomedial nucleus labelled cells in the intralaminar, midline and reticular nuclei as well as showing strong projections from the reticular formation in the brain stem and from nuclei in the floor of the fourth ventricle. (The discussion of the extensive cortical projections will not be included in this presentation).

The findings of differences in the afferents to these two thalamic nuclei might be expected in the case of the dorsomedial nucleus due to its known afferents being very extensive while the medial pulvinar has been reported to have only sparse input. The findings of the input from the reticular, intralaminar and midline nuclei into the dorsomedial nucleus has not been previously reported. We have not yet seen any overlap in the afferent supply to these two functionally different nuclei. We might expect that additional studies will show either the reticular or the midline-intralaminar nuclei having overlapping projections onto these nuclei or that they will remain with separate interthalamic afferents.

351.7 FIBER TERMINALS OF THE SUPERIOR COLLICULAR PROJECTION INTO THE PARAFASCICULAR COMPLEX IN THE RAT. D. S. Yamasaki, and G. M. Krauthamer. Dept. of Anatomy, UMDNJ-Rutgers Med. Sch., Piscataway, NJ 08854.

The tectothalamic input to the parafascicular complex (Pf) of the rat was studied by means of orthograde transport of horseradish peroxidase (HRP). Single and multiple (n_{max}=12) HRP injections were made iontophoretically into the intermediate and deep tectal laminae. The injections were placed in the lateral two-thirds of the superior colliculus, where retrogradely filled tectal cells were found after HRP injections in Pf. The distribution and morphology of these tectothalamic fibers and their terminals were studied at the light microscopic level with cobalt-intensified diaminobenzidine (DAB) as the chromagen.

With both massive and small tectal injections, a high density of fiber swellings (indicative of synaptic boutons) were found throughout Pf ipsilaterally. Most of these fibers were thin and ran in a rostro-dorso-medial direction at caudal Pf levels to arborize at more anterior levels of Pf. Few swellings were found in fibers of passage coursing rostrally through Pf. In each case, fibers projected rostral to Pf into the lateral portion of the medialis dorsalis nucleus (MD) and adjacent parts of n. centralis lateralis (CL). However, the number of fibers and terminal swellings in this region dropped dramatically in comparison to those found in Pf. The labelled fibers from massive tectal injections overlapped the area outlined by retrogradely filled cells following massive HRP injections in the neostriatum of the rat. No topography was found with small tectal injections.

These results suggest that there is considerable communication between the intermediate and deep tectum and the Pf complex in the rat, and that these fibers are not mainly passing through en route to more rostral nuclei.

Supported by NS 10922 and GRS 27-1998.

351.8 ACETYLCHOLINESTERASE-RICH PROJECTIONS TO NEOCORTEX: II. AREAL DISTRIBUTION, LAMINAR STAINING & BARREL INNERVATION STUDIED WITH LESIONS. R.A. McGowan, Jr.,* J. Solomon,* N. Martin-MacKinnon* and D.A. Kristt (SPON: M.E. Smith). Div. of Neuropathology, Stanford University, Stanford, CA 94305.

A major input to neocortex derives from large acetylcholinesterase (AChE)-rich neurons of the basal forebrain. Since the extracortical pathways, intracortical fiber distribution (areal and laminar) were incompletely described in previous work, this study was undertaken. A description of these pathways based on direct observations is presented in a companion abstract. In the present studies, immature and adult rats underwent lesions of one of the three pathways. (These pathways are: (I) Precallosal;(II) Medial and (III) Lateral.) Intra and extra-cortical knife cuts and electrolytic lesions were made and animals were sacrificed 1-4 days later. Brains were processed for AChE histochemistry, as previously described (Kristt, D.A., J.Comp. Neurol. 186:1-16, 1979), Bielchowsky and Nissl stains. Single intracortical lesions that did not involve cingulate cortex (medial pathway), even extensive ones, had relatively little or no effect on AChE staining in neocortex. Multiple knife cuts in different planes had to be made for effects to be noted. Generally, it was also necessary for the lesions to be relatively small, U-shaped or circular. Intracortical knife cuts that produced local reductions in AChE staining needed to extend through layer VI. Coronal lesions at most levels that extended to the midline, through cingulate cortex, produced caudal reduction in staining within cingulate and occipital cortical fields. Extensive extracortical lesions in one of the pathways had the following results: (i) precallosal pathway lesion - reduced AChE in polar cortex, (ii) lateral pathway lesion - AChE reduction in lateral frontal, somatosensory and temporal cortex. These latter lesions resulted in marked generalized loss of AChE staining over extensive areas of cortex. Also, they resulted in almost complete loss of staining in the subpial (layer I) and considerable reductions in the midcortical bands (layers IV and V). However, in immature rats the AChE-positive fiber clusters innervating the SmI barrels were not affected. Some of the tentative conclusions from these data include: (i) there is support for the areal distribution of the basal forebrain pathways suggested in the companion abstract,(ii) fibers in each pathway have an individualized course, (iii) most fibers run for a limited extent in layer VI and then branch radially in a terminal zone with a relatively narrow tangential extent, (iv) there is considerable multi-directional overlap of each of these small terminal domains in neocortex. Support: NSF BNS 81-40895.

351.PO ACETYLCHOLINESTERASE-RICH PROJECTIONS TO NEOCORTEX: I. PATHWAYS FROM BASAL FOREBRAIN & PATTERNS OF DISTRIBUTION. D.A. Kristt. Div. of Neuropathology, Stanford University, Stanford, CA 94305.

The spatial organization of the component pathways of the acetylcholinesterase (AChE)-rich projection system from basal forebrain to neocortex has been traced in the rat and mouse brain. Three main pathways were identified using a histochemical/Golgi approach (Kristt, J.Comp. Neurol.186:1, 1979). The precallosal-sagittal pathway (I) innervates frontal polar cortex. It consists of bundles of AChE-positive fibers arrayed in a curved sheet surrounding the forceps minor of the corpus callosum. These bundles run rostrodorsally along the grey-white junction of polar cortex. Individual fibers can be seen to pass into the forceps minor and/or radiate into the cortex rostral and dorsal to the forceps. Some fibers curve abruptly, leave the sagittal plane of their bundle and turn approximately 90° to run coronally in a lateral to medial direction in layer VI. In coronal sections, these fibers can be followed to approximately 1mm from the midline (adult). Fibers appear to enter the precallosal bundles from two directions mainly. One component of the precallosal bundles represents fascicles of fibers that pass through the striatum (precallosal radiations). The second component derives rostroventrally. Fibers comprising the medial-sagittal pathway (II) can be traced from the region of the diagonal band. The fibers form a tight bundle that runs at first ventral to dorsal in layer VI of medial cortex close to the midline. Some fibers originating from more lateral groups, (possibly from the nucleus basalis/substantia innominata) seem to join this bundle. When the bundle reaches the point where the cortical mantle continues laterally, these fibers turn abruptly caudad. The bundle remains mostly intracortical and supracallosal throughout its course and innervates most of cingulate cortex. Caudally, some fibers veer off laterally from the bundle and pass into the region of the adjacent visual cortex. The lateral-coronal pathway (III) represents a sheet-like array of arcing fibers that innervates lateral frontal, parietal, and temporal cortical fields. Fascicles of AChE-stained fibers emanate from the region of the nucleus basalis, pass laterally, and enter layer VI, turn abruptly dorsomedially, and then coalesce into dense bundles. At levels anterior or posterior to barrel cortex (SmI) the fibers run in coronal planes progressively tilted towards the horizontal. The territories of distribution for each of these pathways is supported by lesion studies (see companion abstract). It is concluded that the pathways to neocortex followed by fibers originating in the basal forebrain are quite varied. Most fibers follow individualized routes to their zones of termination in cortex. Support: NSF BNS 81-40895.

352.1 **THEORETICAL DEVELOPMENT OF BINOCULAR RECEPTIVE FIELDS IN CAT VISUAL CORTEX.** Michael A. Paradiso (SPON: J. Anderson). Center for Neural Science, Brown Univ., Providence, R.I. 02912

When kittens are reared with strabismus the normally binocular receptive fields (RF) of cells in visual cortex become monocular. The usual explanation of this phenomenon is that one eye comes to dominate the cortical cell because of asynchronous activity from the two eyes. However, cells in normally-reared animals also receive uncorrelated activity when their left- and right-eye RFs are not in register. Using a theoretical model we have examined the effect of this asynchrony on the development of binocular receptive fields. We assume that visually inexperienced cells receive varying degrees of uncorrelated input, probably because their RFs are in register at different distances from the plane of fixation. Cells with RFs in register behind or in front of the horopter receive more uncorrelated activity than cells maximally responsive at the horopter. We assume that synapses modify in a manner similar to that proposed by Hebb, as developed by L.N. Cooper. Mathematical analysis and computer simulations yield the following results:

1) Cells which receive a high degree of correlated activity remain binocular and become selective for zero disparity whereas cells which receive more uncorrelated activity become relatively monocular and selective for nonzero disparities. As in strabismic animals, very large amounts of asynchronous activity cause a loss of all binocularity. These results are in agreement with studies of disparity-selective neurons which suggest that cells with disparity are more monocular than cells with zero disparity.

2) For a particular angular disparity, larger RFs are more likely to produce correlated activity and remain binocular. This may explain why in cat binocularity increases with eccentricity from the area centralis and why in strabismic animals complex cells tend to retain binocularity more than simple cells.

3) Because many cells receive some amount of asynchronous activity, any innate ocular dominance preference will be accentuated during development. This may account for the considerable "sharpening" of ocular dominance columns that is observed during the critical period, especially in layer 4.

Thus, with few assumptions about the nature of synaptic plasticity, this model can account for several aspects of normal RFs. It suggests that binocularity will be disrupted by any experimental procedure that decreases RF correspondence. Furthermore, it suggests that the same mechanism that produces an ocular dominance shift in strabismic animals causes there to be a relationship between ocular dominance and disparity in some cells in normal animals.

This work was supported by ONR contract N00014- 81-K-0136.

352.2 STABILITY OF 6-HYDROXYDOPAMINE UNDER MINIPUMP CONDITIONS. Mark F. Bear, Robert J. Clinton, Jr.* & Dean A. Haycock. Brown University, Providence, Rhode Island.

Kasamatsu and Pettigrew pioneered the use of osmotic minipumps to deliver continuously the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) to kitten visual cortex. They found that 6-OHDA infusion for 7 days was sufficient to prevent the normal plastic response to brief periods of monocular deprivation. However, other methods of catecholamine depletion have failed to abolish the ocular dominance plasticity in striate cortex. These findings have led us to re-examine the effects of chronic cortical infusion of 6-OHDA. Because 6-OHDA is known to oxidize rapidly in water, we were interested to know how stable this drug is under minipump conditions at 38°C for 7 days.

4 mM 6-OHDA-HBr (Sigma) was prepared in saline containing 0.4% ascorbate. One aliquot was frozen, the other was stored in a sealed vial in a dark 38°C oven. Aliquots of frozen, warm, and freshly prepared 6-OHDA were monitored for up to three weeks using HPLC with electrochemical detection (ECD). Fresh 6-OHDA yields a peak on the HPLC-ECD with a retention time (22.5 min.) that is clearly distinct from the ascorbate peak (4.4 min.). The peak height (detector response) is linearly related to the amount of 6-OHDA applied to the column, and neither completely oxidized 6-OHDA nor ascorbate gives a peak with this retention time. We found no decline in the detector response to the 38°C 6-OHDA for the first 7 days. However, by day 12 the 38°C 6-OHDA peak was decreased significantly as compared with both the frozen and freshly prepared aliquots. By the 21st day, the 6-OHDA stored at 38°C yielded peaks as low as 10% of the frozen sample.

To confirm that the HPLC peaks were an accurate reflection of the drug's effectiveness, we injected mice i.v. with 7-day-old 38°C 6-OHDA (20 mg/kg) and compared the depletion of heart norepinephrine (NE) with that obtained with freshly prepared 6-OHDA. Whole heart NE was reduced to 10±1% of control in mice injected with fresh 6-OHDA (N=8) and to 14±3% of control in animals injected with 7-day-old 6-OHDA (N=3). Thus, 4 mM 6-OHDA stored at 38°C is still an effective neurotoxin for at least 7 days.

We conclude: 1) 6-OHDA may be assayed with HPLC-ECD and 2) 4 mM 6-OHDA-HBr in 0.4% ascorbic saline is stable under minipump conditions for at least 7 days. Our results confirm that 6-OHDA, not a breakdown product, is likely to be the agent that disrupts plasticity in kitten visual cortex.

We acknowledge the support of Drs. R.L. Patrick & F.F. Ebner and of ONR contract N0014-81-K-0136.

352.3 PERMANENT DEFICITS IN BINOCULAR DEPTH PERCEPTION DUE TO LARGE OPTICALLY-INDUCED INTEROCULAR TORSIONAL DISPARITY IN EARLY VISUAL INPUT. Michael R. Isley*, Brian Timney, Diane C. Rogers*, and Paul G. Shinkman. Univ. North Carolina, Chapel Hill, NC 27514, and Univ. Western Ontario, London, Canada N6A 5C2.

Kittens were reared wearing goggles fitted with small prisms that introduced equal and opposite rotations of the two eyes' visual fields about the visual axes; the rotations were 16° in each eye for a total torsional disparity of 32°. The kittens received 100-150 hr of visual experience starting at 4 weeks of age and continuing for 1-2 months, and remained otherwise in the dark. Control kittens wore goggles with prisms that introduced 0° of interocular rotational disparity. As we have reported previously, this manipulation produces important changes in the physiological organization of visual cortex, most notably a severe disruption of binocularity, with ocular dominance histograms that are U-shaped rather than normal. Furthermore, these effects are permanent; when 32° goggle kittens are returned to the colony and then retested neurophysiologically after 4-8 months of normal visual experience, no further changes are observed.

We now report the behavioral consequences of 32° prism goggle rearing. There is a severe disruption of binocular depth perception. Using the jumping stand technique, these kittens are unable to make more than the grossest of depth discriminations. The deficit is not due to poor acuity, which is near the lower limit for normally reared kittens. It is also not due to rearing artifacts, since control kittens exhibit binocular depth perception in the low normal range, and substantial superiority in binocular as compared with monocular performance. Furthermore, paralleling the physiological effects, the perceptual deficit is also permanent. Two kittens were reared as described above, and showed the expected cortical effects. After 3 yr of normal visual experience, they exhibited extremely poor binocular depth discrimination, with depth thresholds 4-5 times greater than normally reared kittens. This finding is consistent with other recent reports showing little or no recovery of binocular depth perception in monocularly deprived kittens, even after extensive subsequent normal visual experience. We conclude that when the capacity for binocular depth perception is lost early in life, normal experience does not lead to recovery of this perceptual capability.

Supported by USPHS grants MH-14269 to P.G.S. and HD-03110 to the Biological Sciences Research Center, ONR Contract N00014-83-K-0387, and the Medical Research Council of Canada.

352.4 GAMMA-AMINOBUTYRIC ACID (GABA) RECEPTORS IN VISUAL CORTICAL CELLS OF NORMAL AND DARK REARED KITTENS. K. Tanaka, R.D. Freeman and A.S. Ramoa.* Sch. Optom., Univ. of Calif. Berkeley, CA 94720.

During the first postnatal month of the kitten, substantial developmental changes occur in geniculocortical and intracortical transmission. Concomitantly, responses of some proportion of cortical cells become stimulus-specific with respect to orientation, direction, and/or disparity selectivity. This stimulus specificity fails to develop completely or degenerates in dark-reared kittens. Since orientation and direction selectivity in the adult cat appears to be dependent upon intracortical GABAergic inhibitory connections, we hypothesized that these connections develop postnatally, and fail to develop or atrophy in dark-reared kittens. To test this idea, we examined a component of the inhibitory process by measuring effects of locally applied GABA on impulse activity of cells in the visual cortex of normal 4 and 8 week old kittens and adults and of 9 and 10 week old dark-reared kittens.

A three-barreled glass electrode was positioned into area 17. A tungsten wire was inserted into one of the barrels to record impulses of single cortical cells extracellularly. The other two barrels were filled with DL-homocystein (DLH,0.5M,pH7.5), GABA (0.5M,pH3) or glycine (0.5M,pH3). DLH was used to increase spontaneous activity and glycine was used in place of GABA for some measurements as a control procedure to determine if observed effects were GABA-specific. We plotted receptive fields and determined if a cell was orientation selective, biased, non-oriented or visually unresponsive. Then GABA or glycine was iontophoretically applied and currents were determined which were sufficient to suppress impulse discharges elicited by optimal visual stimuli and also by iontophoretically applied DLH.

Nearly all cells in the adult and 8 week old normal visual cortex demonstrated stimulus specificity as expected. Application of GABA invariably suppressed discharges from these cells. Specificity was not fully exhibited in 4 week old kittens but discharges of cells which were not orientation selective were suppressed with GABA as effectively as in the case of orientation-specific cells. The majority of cells in the dark-reared kittens were non-oriented, visually unresponsive or orientation biased and these cells were also suppressed with GABA in a manner not distinguishable from orientated cells. The only difference we noted for these latter two groups was a tendency for less required current to suppress discharge activity. In all cases, glycine was relatively ineffective in suppressing discharges. We conclude that GABA receptors are functional at the postsynaptic cell level in young and dark-reared kittens.(EY01175)

352.5 **FUNCTIONAL ORGANIZATION OF THE CAT'S VISUAL CORTEX AFTER PRENATAL UNILATERAL ENUCLEATION**

Brenda L. Shook*, Lamberto Maffei*, and Leo M. Chalupa
Department of Psychology, University of California, Davis, CA. 95616.

Ocular dominance and orientation columns are two basic characteristics of the organization of the visual cortex of carnivors and primates. It has been demonstrated in the monkey that prenatal binocular competition plays a critical role in the formation of ocular dominance columns (Rakic, 1981). Removal of one eye in the fetal monkey results in a continuous projection pattern from the remaining eye to layer IV of the striate cortex. The aim of the present experiment was to determine whether such a widespread geniculocortical projection occurs in cat after in utero eye removal. We also sought to examine the physiological properties of visual cortical neurons in prenatal enucleates. We were particularly interested in determining whether the sequence of orientation columns developes normally following termination of in utero binocular competition.

Experiments were performed on adult cats, enucleated more than two weeks before birth or at maturity. Results from these animals were compared to those obtained from normal adult cats.

Anatomy. The anatomical organization of geniculocortical projections was investigated by making ion tophoretic injections of WGA-HRP into single lamina of the dorsal lateral geniculate nucleus. In prenatally enucleated cats injections into the superficial portion of the dorsal lateral geniculate (A of the normal adult cat) resulted in a continuous band of label in layer IV of areas 17 and 18. Comparable injections made in adult enucleated cats resulted in a patchy but graded band of label in layer IV of areas 17 and 18. These patches appeared to be wider than those which were found in normal controls.

Physiology. Tangential penetrations through area 17 in the prenatally enucleated cats showed regions of continuous neuronal activity. These pen etrations, some as long as 3 mm, traversed a region of cortex which in the normal cat would have been occupied by a number of discrete ocular dominance columns. The topography of receptive fields was normal and no discontinuities in field progression were noted. Nor were any visually unresponsive or poorly responsive cells encountered. Furthemore, neurons exhibited an orderly progression in orientation selectivity. In some penetrations we isolated a sequence of neurons containing a full 180° cycle of changes in preferred ori entations. Such an orderly 180° cycle of changes in preferred orientations in the normal animal define a hypercolumn. In comparison, cats enucleated as adults showed somewhat elongated "dominance columns" separated by smaller silent zones and regions which contained spontaneously active but poorly responsive cells.

We conclude that within regions sampled in this study, the topography and distribution of preferred orientations in the prenatally enculeated cats are similar to those which are observed in intact controls. The development of orientation columns seems to be independent of the formation of ocular dominance columns.

Supported by NEI grants EY05670 (B.L.S.) and EY03991 (L.M.C.)

352.6 CORTICAL EFFECTS OF SHORT PERIODS OF MONOCULAR DEPRIVATION.

M.A. McCall* and H.V.B. Hirsch, Departments of Ophthalmology and Psychology, Univ, of Madison, Madison, Wisconsin 53706 and Center for Neurobiology, SUNYA, Albany, NY 12222.

In order to look for differences in sensitivity to monocular deprivation (MD) among cells in the visual cortex (area 17) of the cat we studied effects of brief (4-10 day) periods of MD which were sufficient only to result in partial take over of cells by the non-deprived eye. Cats were subjected to MD at different ages (9-100 days of age) to assess possible age-related changes in the effects of MD. The animals were given normal, binocular visual exposure until the beginning of the MD. We recorded extracellularly from single units in area 17 in 19 cats immediately after the MD and in 10 normal cats of similar ages. The following response properties of cortical cells were measured: evoked activity, spontaneous activity, receptive field size, cutoff velocity, orientation selectivity and ocular dominance.

In both normal and MD cats there were comparable differences between monocular and binocular cortical cells: monocular cells had significantly smaller receptive fields, were more finely tuned for stimulus orientation and had lower spontaneous activity levels than did binocular cells. While the proportion of monocular cells was higher in MD cats than in normal cats, we found that MD did not produce a change in the distribution of the cell types activated by the two eyes: there were no differences in response properties between cells activated by the deprived eye and cells activated by the non-deprived eye at any of the ages tested. We thus found no evidence for a preferential take over of a particular cell type by the non-deprived eye. Supported by NEI Grant EY01268 to H.V.B.H .

352.7 DO SHORT TERM AND LONG TERM DEPLETION OF NORADRENALINE HAVE DIFFERENT EFFECTS ON VISUAL DEPRIVATION IN THE KITTEN VISUAL CORTEX?

N.W. Daw, R.K.Rader*, T.W. Robertson* and T.O. Videen*. Physiology Dept., Washington University Medical School, St. Louis, MO 63110.

Various methods have been used to deplete noradrenaline and to observe whether this influences the effects of monocular deprivation in the kitten. Normally monocular deprivation leads to a shift in the ocular dominance histogram, with most cells dominated by the open eye. This situation is unchanged by electrolytic lesions of the dorsal noradrenergic bundle, 6-OHDA lesions of the dorsal noradrenergic bundle, neonatal intraperitoneal injections of 6-OHDA and 6-OHDA lesions of the locus coeruleus, all of which, inter alia, deplete noradrenaline in the visual cortex by 70-90%. However, after injections of 6-OHDA directly into the visual cortex, combined with monocular deprivation, the ocular dominance histogram is much flatter; after injections of 6-OHDA into the ventricle the results are mixed.

One suggestion which might make these results more compatible with each other is that the flat ocular dominance histogram occurs after short term depletions of noradrenaline while the shifted ocular dominance histogram occurs after long term depletions, with the possibility of compensatory mechanisms occurring. We therefore decided to compare short term and long term depletions, using the drug DSP-4. Seven kittens were given the drug injected intraperitoneally (20-30 mg/kg) at approximately four weeks of age. In four kittens one eye was sutured 2-4 days later. In the other three one eye was sutured 10, 14 and 17 days later. Single unit responses from the visual cortex were recorded approximately 10 days after the eye suture. In all cases the ocular dominance histogram was dominated by one eye and the delay between DSP-4 injection and eye suture did not affect the result. The concentration of noradrenaline in the visual cortex was measured by HPLC and was depleted by 75-90% compared to normal kittens of the same age. The addition of one more method to the list of treatments which deplete noradrenaline but do not change the effects of eye suture raises further doubts about the interpretation of the effects of intracortical and intraventricular administrations of 6-OHDA.

352.8 DEVELOPMENT OF ACETYLCHOLINESTERASE ACTIVITY IN LAYER IV OF RAT VISUAL CORTEX: NORMAL DEVELOPMENT AND THE EFFECT OF NEONATAL MONOCULAR ENUCLEATION.

Richard T. Robertson and Alberto A. Tijerina.* Department of Anatomy, College of Medicine, University of California, Irvine, CA 92717.

The distribution and possible function of acetylcholinesterase (AChE) in cerebral cortex have recently attracted a great deal of interest. While much attention has been focussed on the system of AChE-positive cortical afferents from the basal forebrain, it appears that other AChE-positive systems are also present in cortex. We report here the development of AChE activity in layer IV of rat visual cortex and the effects of neonatal monocular enucleation.

Subjects were laboratory born male or female Long-Evans hooded rats of 0-45 post-natal days of age. AChE activity was detected histochemically in series of 50 um frozen sections by a modified version of the method of Koelle. Non-specific cholinesterase activity was inhibited by 10^{-4} M iso-OMPA. In some cases butyryl-thiocholine was used as the substrate.

In normal juvenile and young adult rat (post-natal day 30-90) AChE reaction product in visual cortical area 17 occurs in a fiber-like plexus that is particularly dense in layer IV. The appearance of this dense AChE plexus resembles an axonal terminal field. The plexus is co-extensive with the terminal field of geniculo-cortical projections and does not extend beyond cortical area 17 into area 18 or 18a.

AChE activity first appears in area 17 as a fine fiber-like plexus in layer IV at about 7 post-natal days of age. The density of the staining increases remarkably during days 11-19 and reaches the young adult pattern by about day 25.

Neonatal monocular enucleation results in a marked decrement in the development of AChE activity in cortical area 17 contralateral to the enucleated eye. An AChE-positive plexus is still found in layer IV, but its areal extent is more restricted than in the visual cortex ipsilateral to the enucleated eye and the density of the AChE reaction product is decreased.

These data indicate that normal patterns of AChE activity in rat visual cortex are dependant on normal afferent innervation and/or stimulation.

Supported by NSF grant 79-14223 to RTR.

352.9 HUMAN VISUAL SYSTEM: GLOBAL RIGIDITY OF THE SENSORY MAP - LOCAL PLASTICITY WITHIN THE SENSORY MAP - GLOBAL PLASTICITY OF THE OCULOMOTOR MAP. P. Stoerig*, E. Pöppel and W. Fries*. (SPON: H. Hollaender). Institute of Medical Psychology, University of Munich, FR Germany.

A patient who since early childhood had a 17° convergent squint of his left eye suffered a left occipital lobe lesion. The areas of blindness in the two eyes were not homonymous. Whereas perimetry of the right eye showed an area of blindness with the vertical meridian as a border, the left eye's blindness was different: The unimpaired visual field extended by approximately 17° into the nasal visual field. The blind spot of the left eye was observed next to the fixation point in the nasal half-field. When the vertical borders of the two eyes are superimposed, the lateral shift of the vertical meridian amounts to 17°. This observation indicates that the cortical lesion results in an area of blindness in the visual field of the left eye with respect to the anatomical fovea, not with respect to the "pseudo-fovea". Thus, the central representation of the visual field is rigid; 55 years of misaligned visual input seem to leave the prewired map untouched. Plastic changes within the map were, however, observed after measuring increment threshold. The left (squinting) eye shows a decrease of threshold between the fixation point and the visual field position corresponding to the anatomical fovea - an indication of rigidity. The right (normal) eye, however, shows an increase of threshold by more than half a log.-unit at the visual field position corresponding to the blind spot of the left eye. As peripheral effects are unlikely to account for this effect, an interaction of non-corresponding retinal positions is very likely. In addition to such local plasticity within the visual map, there is global plasticity within the oculomotor system. If the right eye is covered, the left eye makes saccades of proper direction and amplitude using the fixation axis as reference. Thus, it is no longer the anatomical fovea of the left eye which serves as reference for defining left and right, but the acquired pseudo-fovea. Contrary to the rigidity of the sensory map, there is a recalibration of the oculomotor map, adjusting the left to the right eye. (Supported by grant Po 121/10 of the DFG).

352.10 DEAFFERENTATION OF THE VISUAL CORTEX: THE EFFECT ON CORTICAL CELLS IN NORMAL AND IN EARLY MONOCULARLY DEPRIVED CATS. U. Yinon* M. Podell* and S. Goshen*(SPON : PROF. Z. Elazar). Physiological Lab., Goldschleger Eye Inst., Tel-Aviv Univ. School of Medicine, Sheba Medical Center, Tel Hashomer 52621, ISRAEL.

The optic tract was unilaterally sectioned and receptive field mapping and unit recording were made for cells in the boundary of areae 17-18 in the deafferented and in the intact visual cortex of adult cats monocularly deprived during the critical developmental period. Three groups of adult animals served as controls: normal cats, early monocularly deprived (MD) cats and optic tract sectioned cats. In contrast to the activity found in the intact hemisphere the deafferented hemisphere of the experimental group was almost completely unresponsive. The ocular dominance distribution in the intact hemisphere of the experimental group (75.0% monocularly driven cells) was similar to that of the control MD cats (81.5% monocularly driven cells). This indicates the absence of a specific post critical period effect of visual isolation of one hemisphere on the monocular dominance early induced in the intact hemisphere. However, the reduction found in the proportion of visually responsive cells and orientation and direction selective cells in the intact hemisphere of the experimental group, is mainly due to the isolation of the fellow hemisphere from its direct visual input, and the subsequent inactivation of the callosal pathway interconnecting the two visual areas.

352.11 CHANGES IN PROTEIN SYNTHESIS IN THE VISUAL PATHWAY REFLECT PLASTICITY IN DEVELOPING MONKEY VISUAL SYSTEM. C.Smith*, A.Crane* (SPON: L.Sokoloff). Lab.of Cerebral Metabolism NIMH, Bethesda, MD 20205.

In the monkey the organization of the visual system retains some plasticity during the first six weeks of life. Monocular deprivation during the first three weeks results in a reorganization of the ocular dominance columns in the striate cortex and a change in cell size in some layers of the dorsal lateral geniculate nucleus (dLGN) (Hubel et al., Phil.Trans.R.Soc. Lond.B 278, 377,1977). The processes underlying these plastic changes are undefined. It is thought that domination of a column in the cortex occurs because one set of afferents gains a competitive advantage over the other, a process which may be thought of in terms of trimming and/or sprouting. In this study we have attempted to differentiate between sprouting and trimming by measuring the rates of protein synthesis in the cell bodies located in the dLGN. Growth and sprouting are dependent on protein synthesis, a process confined to neuronal cell bodies. Increased sprouting at the terminals should be reflected in increased protein synthesis in the cell bodies of origin whereas trimming or retraction should be reflected in decreased protein synthesis. Local rates of protein synthesis were measured by a recently developed autoradiographic method (Smith et al., Trans.Am.Soc.Neurochem. 11,94,1980). Whereas acute monocular deprivation had no apparent effects, chronic monocular deprivation in the newborn rhesus monkey resulted in reduced protein synthesis in the dLGN cell bodies of the deprived pathway. Neither acute nor chronic deprivation had any apparent effect on protein synthesis in the striate cortex. Twenty-five days after reverse suture, which was carried out on day 25, the rates of protein synthesis in the laminae of the dLGN had reversed such that the layers subserving the initially deprived eye had higher rates of protein synthesis than the layers subserving the initially open eye. Furthermore, in the striate cortex, columns with alternating high and low rates of protein synthesis were evident. The columns were perpendicular to the cortical surface with a periodicity of about 0.8mm. They were particularly distinct in layers 2-4. The portion of the period with the higher rate of protein synthesis was slightly wider. These results show that in the newborn monkey the reorganization that occurs in response to chronic visual deprivation is reflected in changes in protein synthesis in the cells along the visual pathway. The reduction in protein synthesis in the dLGN may underlie an inadequate maintenance of terminals in the striate cortex with a consequent loss of the competition to afferents from the nondeprived eye. The results of the reverse suture experiment suggest that the reorganization of the visual cortex that takes place in response to this experimental procedure also involves changes in protein synthesis in cortical cells.

352.12 STEREOBLIND MONKEYS HAVE FEW BINOCULAR NEURONS. M.L.J. Crawford, E.L. Smith,, III, R.S. Harwerth, and G.K. von Noorden. Univ. Texas Grad. Sch. Biomed. Sci.; Univ. Houston; and Baylor College of Medicine, Houston, TX 77025.

Eight hundred and eighty (880) neurons were recorded from the visual cortices of seven rhesus (M. mulatta) monkeys, three of which had had optically-induced strabismus early in life. These monkeys had no detectable strabismus for a 3-year period following only 30 days of viewing the world through prisms. Prior extensive behavioral testing showed that even though they were not amblyopic, they were stereoblind to dynamic random dot stereograms (Crawford, M.L.J., et. al., Invest. Ophthal. & Vis. Sci., 24:491-495, 1983).

The eye-dominance profiles for samples of neurons recorded from the striate (V1) and from the pre-striate (V2) cortices were normal in all regards except one, the degree of binocularity. In control monkeys, (fig. 1) 81% of the cortical neurons had binocular input, compared to only 22% (Fig. 2) in the experimental monkeys.

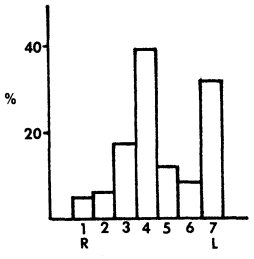


fig. 1

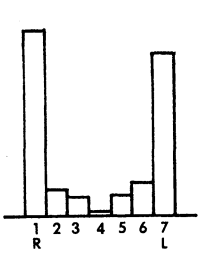


fig. 2

These results: (1) Demonstrate for the first time the association between cortical binocular neurons and primate stereopsis. (2) Show that binocular striate neurons, once lost do not recover even with extensive binocular visual experience and (3) Stress the sensitivity of the binocular system to abnormal early visual experience.

(Supported by NIH Grants EY01120 and EY02520).

353.1 CORTICO-CORTICAL CONNECTIONS IN THE RAT SUBSERVING VISUOSENSORY AND VISUOMOTOR INTEGRATION. Michael W. Miller and Brent A. Vogt. Dept. of Anat., Tulane Univ. Sch. of Med., New Orleans LA 70112 and Dept. of Anat., Boston Univ. Sch. of Med., Boston MA 02118.

Direct connections of rat visual cortex were examined by tracing the anterograde transport of tritiated amino acids or the retrograde transport of horseradish peroxidase conjugated to wheat germ agglutinin from pressure injections placed in area 17, 18a, or 18b.

Visual cortex is connected reciprocally with visual, somatosensory, and auditory cortices. Areas 17, 18a, and 18b are interconnected with each of the visual areas. Secondary visual cortices have direct connections with primary somatosensory cortex. Following injections in area 18a, autoradiographic label or HRP-positive neurons are evident in posterior central area 3. On the other hand, area 18b is connected with a locus in posterior, medial, area 3. Both secondary visual areas are connected with the dorsal one third of area 41, primary auditory cortex, as well as with dorsal temporal area 36, secondary auditory cortex.

Each visual area projects to and receives afferents from motor area 8. In fact, the projections from areas 17, 18a, and 18b overlap in the posterior one third of area 8.

Visual cortex also has extensive connections with association cortices. Areas 17, 18a, and 18b are connected reciprocally with area 7, that part of parietal cortex which is directly anterior to secondary visual cortex. This connection is organized topographically; area 18b is connected with medial area 7, area 17 with central area 7, and area 18a with lateral area 7. Each visual area also has strong, reciprocal connections with posteroventral area 36 and is interconnected with perirhinal areas 13 and 35. Secondary visual areas have additional reciprocal projections to and from association areas. Area 18a is connected with ventral parietal areas 39, 40, and 14. Area 18b has a unique and strong interconnection with area 11 or "orbital" cortex, the region of dorsal frontal cortex which is adjacent to the olfactory bulb. It is interesting to note, that only area 18b is interconnected with the claustrum.

These pathways and others described may provide the basis for the visuosensory and visuomotor integration which aid the rat in coordinating visually guided behaviors. For example, the parts of somatosensory cortex which are connected with secondary visual cortex correlate with the eye, eyelid, and neck representations, and stimulation of posterior area 8 produces movements of the eyes and eyelids.

This research was supported by N.I.H. grants NS 07016, EY 07054, and NS 18745.

353.2 PROJECTIONS OF THE ANTERIOR ECTOSYLVIAN VISUAL AREA UPON AREAS 17/18 AND LATERAL SUPRASYLVIAN IN THE CAT AS DEMONSTRATED BY THE RETROGRADE MULTIPLE-LABEL FLUORESCENCE TECHNIQUE. D. Miceli, R. Gagnon, M. Ptito* and J. Repérant. Groupe de recherche en neuropsychologie expérimentale, Université du Québec, C.P. 500, Trois-Rivières, Québec, G9A 5H7, CANADA. Laboratoire de psychophysiologie sensorielle, Université de Paris VI, Paris, Cedex 05, France.

Recent physiological and anatomical investigations have identified a specific visual area within the ventral bank of the anterior ectosylvian sulcus (AES) in the cat (Mucke et al., 1982, Roda and Reinoso-Suarez, 1983). The aim of the present study was to determine possible interrelations between AES and other cortical visual areas using the retrograde multiple-label fluorescence technique. Retrogradely-labeled neurons were mapped in AES following unilateral injections of 0.1 - 0.3 uL of different fluorescent tracers (Fast Blue: 5% w/v, Nuclear Yellow: 3%, Evans Blue: 10% solution containing 1% poly-L-ornithine) into the lateral suprasylvian area (LS: involving PMLS and PLLS) and the border regions of areas 17/18 and 18/19. Neurons labeled with the tracer injected into LS were indentified bilaterally within the ventral bank and fundus of AES. The labeling was homotopic, predominantly ipsilateral and localised within layer III but more extensively in layer VI. Relatively fewer fluorescent cells containing the tracer injected into areas 17/18 were also detected bilaterally within the fundus region of AES and restricted to the deeper portion of layer VI. Although some overlap in the distribution of the differentially labeled cells was evident in the latter layer, double-labeled neurons which would indicate collateralised axonal connections with areas 17/18 and LS were never observed. Finally, no labeling of cells with the tracer injected into areas 18/19 was apparent in AES. The present fluorescence results demonstrate efferent projections of AES upon both geniculate- and extrageniculate-recipient visual cortical areas. Although the AES projection upon LS is prominent, its direct connections with striate/peristriate visual cortices (areas 17/18) suggest a more complex functional role in visual behavior.

This work was supported by CRSNG research grants A7912 and E5641.

353.3 AREA 17/18 FIBRES PROJECTING TO LATERAL SUPRASYLVIAN CORTEX RELEASE EXCITATORY AMINO ACIDS. W.D. Ruwe, T.P. Hicks, L. Bauce* and W.L. Veale. Depts. of Medical Physiology and Anatomy, Lions' Sight Centre, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Neurones in the lateral suprasylvian area (LSA) of cats are visually responsive. They receive this sensory information by way of synaptic input from cells in the pulvinar, lateral posterior nucleus, tectum and from a variety of cortical sites, including the border region between areas 17 and 18. It has recently been shown (Hicks, T.P. and Guedes, R.C.A., Exp. Brain Res., 49: 157-173, 1983) that antagonists of the excitatory amino acids selectively block the responses of LSA cells evoked synaptically when other cortical sites projecting to the LSA are stimulated. This prompted the suggestion that either glutamate or aspartate might function as a synaptic transmitter of these cortico-cortical projections. The present experiments were undertaken in order to examine this possibility through the use of the push-pull cannula technique, combined with high performance liquid chromatography (HPLC) for the detection and measurement of suspected transmitter substances.

Cats were anaesthetized with ethyl carbamate (1.6 g/kg.) or α-chloralose (70 mg/kg.). Electrical stimuli were delivered to cells in the 17/18 border region through concentric bipolar electrodes (40 V. stim; 20 Hz of 5 sec. on - 5 sec. off; 1-2 msec duration). The LSA was perfused with artificial CSF containing 5 mM glucose before, during and after the electrical activation of visual area 17/18. A stainless steel guide tube was implanted proximal to the LSA. To perfuse a site within this area, the push-pull cannulae were lowered through this guide tube. The rate of perfusion was 30.0 µl/min and each perfusion was 30 min in duration. An interval of 5 min elapsed before commencement of a subsequent perfusion. Usually, a series of 9 perfusions were conducted in each experiment: (a) 3 control perfusions prior to delivery of the electrical stimuli; (b) 3 perfusions during the electrical stimulation; and (c) 3 perfusions following stimulation, to obtain a recovery sequence. Resting levels of glutamate released into the perfusate were slightly more than twice those of aspartate. During electrical stimulation of the 17/18 border area, release of aspartate rose over twelve-fold, while glutamate levels increased fourteen-fold. These enhanced values approached normal following the period of electrical stimulation (recovery sequence). These data further support the proposal that an excitatory amino acid functions as a synaptic transmitter of the 17/18 projection to the LSA.

(This work was supported by the Alberta Heritage Foundation for Medical Research and the MRC of Canada).

353.4 CURRENT SOURCE DENSITY ANALYSIS OF 16-CHANNEL VEP IN ALERT MONKEY STRIATE CORTEX SUGGESTS EXTRASTRIATE ORIGIN OF THE SURFACE VEP. Manfred Mackeben and Ken Nakayama. Smith-Kettlewell Institute of Visual Sciences, San Francisco, Ca. 94115

Visual evoked potentials (VEPs) recorded over the occipital cortex of humans and monkeys show important similarities, although the visual topography of striate cortex (area V 1) is very different in relation to the brain surface. This indicates that proximity of generators should not be a crucial consideration in the interpretation of VEP recordings.

VEPs were recorded simultaneously in 16 depths of the striate cortex in the alert, trained monkey using a 16-channel linear electrode array permanently implanted in area V 1 of the occipital lobe. Visual stimulation was performed by onset-offset and counterphase flicker of sinusoidal grating patterns. The resulting sets of field potentials were subjected to current source density (CSD) analysis. This procedure extracts activity originating in the tissue directly surrounding the electrodes, where current sinks have been associated with excitatory neural activity (Mitzdorf and Singer, Exp. Brain Res. 33, 1978).

Conspicuous CSD features varied in amplitude, temporal structure and cortical depth, according to the type of visual stimulus used. They did not show a strong dependence on spatial frequency, however. We estimated a component common to all channels, which consists of volume-conducted potentials originating in distant brain loci. This "far-field potential" can be subtracted from the "raw" field potentials, thus giving us an estimate of the "near-field potential". We also compared recordings made in the tissue with surface VEP recordings made with electrodes implanted at the bases of our depth electrodes. We found that the surface VEP differed only very slightly from recordings made in the most superficial layer of the brain. After subtraction of the far-field potential, the VEPs recorded from the superficial layers of the brain (and, thus, the surface VEP) are reduced to very small amplitudes. Furthermore, the far-field potential often constituted a large percentage of the surface potential amplitude and showed spatial frequency tuning characteristics resembling the ones found in surface recordings (Nakayama and Mackeben, Vis. Res., 1982). This suggests that potentials originating in extrastriate visual areas contribute strongly to the surface VEP.

(Supported by NIH grant nos. 1R01 EY 03598, 2EY 01186, BRSG 08-S7RR05566B, and by the Smith-Kettlewell Eye Res. Foundation).

353.5 CORTICAL CONNECTIONS OF STRIATE CORTEX IN TREE SHREWS. M. A. Sesma, V. A. Casagrande and J. H. Kaas. Depts. of Psychology and Anatomy, Vanderbilt University, Nashville, TN 37240.

The tree shrew is a diurnal mammal that is closely related to primates. The well-developed visual system of the tree shrew has been extensively studied, particularly the functional organization of striate cortex. While the connections of striate cortex have been described in some detail in a number of primate species, they have not yet been reported for tree shrews.

We examined the connections of striate cortex in tree shrews using a number of anatomical tracers including ^{3}H-proline, ^{3}H-leucine, ^{3}H-WGA and HRP. In order to reveal the overall pattern of projections, two cases were prepared by flattening the cortex after perfusion and sectioning the block parallel to the flattened surface.

Projections of striate cortex were revealed in three regions of extrastriate cortex. One projection zone was in Area 18 or V-II, which forms a narrow band on the lateral border of striate cortex. Projections to Area 18 were somewhat patchy or discontinuous, and they appeared to conform to visuotopic locations that matched those of the injection sites in V-I. A second projection zone was on the lateral border of Area 18 in part of the cortex sometimes designated as Area 19. Often the label in this second projection zone was continuous with that in Area 18. A third projection zone in more lateral cortex in the temporal lobe was separated from the second projection zone by a 1-2 mm wide zone of unlabeled cortex. Termination zones in all three projection regions extended vertically through most or all cortical layers. Cells in these projection zones filled retrogradely from striate injections were located in layers III and V. The material also allowed observations of the intrinsic connections of Area 17. Around the injection sites, puffs of label similar to those described by Rockland et al. (J. Comp. Neurol., '82) were apparent in layers IIIc and upper layer VI.

The projection pattern of striate cortex in tree shrews resembles that found in primates. All primates appear to have two projection zones, one in Area 18 or V-II, and another in the Middle Temporal Visual area (MT) of the temporal lobe. In some primates projections are found in an additional area or areas bordering V-II. Tree shrew striate cortex has projections to V-II, to a region of temporal cortex that may be a homologue of MT, and to cortex bordering V-II.

Supported by N.I.H. grants EY-02686 (J.H.K.), 1-K04-EY00223, and EY01778 (V.A.C.).

353.6 MYELINATION OF THE VISUAL FIBERS OF THE CORPUS CALLOSUM IN CATS Greg A. Looney and Andrea J. Elberger, Dept. of Neurobiology and Anatomy, Univ. Texas Medical School at Houston, Houston TX 77025.

The time of appearance and development of myelinated callosal fibers in cats was studied in order to correlate development of this fiber bundle with functional maturation. Seventeen cats of known gestational age ranging from 15 to 453 postnatal days (PND) were perfused and segments of the corpus callosum were prepared for electron microscopic analysis. The visual part (splenium) of the corpus callosum was thin sectioned in the sagittal plane and a random sampling method was used to photograph the tissue on the EM. The following parameters were examined in order to assess callosal development: density of myelinated fibers, axonal area, and number of myelin lamellae.

The posteriormost aspect of the splenium begins myelination on PND 18 and the density of myelinated fibers increases up to adulthood. Less than 600 new fibers/mm^2/day myelinate on PND 18 and 19. These fibers averaged 0.66 μm^2 in cross sectional area and 3.7 lamellae/axon. Only 1-2 lamellae were present in 41% of the population and axons were never seen with more than 9 wraps. The rate of recruitment of axons for myelination was highest between PND 21 and PND 24, with more than 10,000 fibers/mm^2/day myelinating then. During this time the mean cross sectional area of myelinating axons increased to 1.1 μm^2 with 6.6 lamellae/axon, and some fibers had already reached the adult stage of myelination. Only 3% of the axons on PND 21, 22, and 24 contained less than 3 lamellae indicating that myelination proceeds rapidly during this period once it begins. The recruitment rate of axons for myelination decreased after PND 24 and gradually dropped to 400 myelinating fibers/mm^2/day between PND 112 and 453. The size of myelinating fibers remains constant between PND 24 and 55, after which the smallest fibers (<0.28 μm^2) myelinate. This size class comprises 36% of the adult myelinated fiber population.

These data indicate that the splenium begins to myelinate by PND 18, and the period PND 21-24 has the highest rate of myelin wrapping as well as the highest recruitment rate of axons for myelination. Furthermore, from PND 21-24 either the myelinated axons are increasing in size or else myelination begins on larger axons than during PND 18 and 19. The first fibers to myelinate are within the smallest third of the size distribution; myelination of the largest fibers peaks between PND 29 and 55, and the very smallest axons begin myelinating by PND 55. Since myelination of the splenium does not begin until the later part of the callosal critical period (Elberger and Smith, this volume) it is unlikely that myelination of the corpus callosum plays a major role in the callosal critical period of visual development.

Supported by NIMH Grant MH36526 awarded to A.J.E.

353.7 CALLOSAL CONNECTIONS OF VISUAL CORTEX IN OWL MONKEYS, MARMOSETS, AND GALAGOS. C. G. Cusick, H. J. Gould, III, and J. H. Kaas. Depts. of Psychology and Anatomy, Vanderbilt Univ., Nashville, TN 37240 and Dept. of Anatomy, LSU Medical Center, New Orleans, LA 70112.

Visual connections of the corpus callosum were studied in adult owl monkeys (Aotus trivirgatus), marmosets (Callithrix jacchus), and galagos (Galago crassicaudatus) by placing multiple injections of horseradish peroxidase in visual cortex of the dorsolateral brain surface, allowing a 2-3 day survival period, and processing the opposite hemisphere with TMB histochemistry (Mesulam, 1978).

For most of the brains, the cortex was removed, flattened between glass plates, and sectioned parallel to the surface so that areal extents of anterograde and retrograde label could be accurately determined. In all three primates, a dense band of terminations and labeled cells was obvious along the outer border of Area 17. Callosal terminations extended into Area 17 where they included layers I, III, and VI. Callosally projecting neurons were located as far as 1 mm within Area 17, and they occurred in layer III. In Area 18, labeled cells and terminations were most dense within 1-1.5 mm from the Area 17 border, although some callosal connections were seen more rostrally in Area 18, and moderate to dense label was seen across the width of Area 18 in lateral cortex where the representation of the 0^{o} horizontal meridian occurs. Labeled neurons in Area 18 were in layer III, with a few in layer V, while terminations were most dense in layer IV. Area MT, which was identified from brain sections stained for myelin, had label throughout, although label tended to concentrate at the border. Variations in density occurred along the border, and within MT. Labeled neurons were mainly in layer III, and terminations were concentrated in layers III and IV. Areas DL, DI, and DM were studied only in owl monkeys, where they could be identified by location. DL had dense callosal connections caudally in correlated to the horizontal meridian. DM had few callosal connections along its caudal border with Area 18, but dense callosal connections occurred rostrally near the representation of the vertical meridian. The region of DI had moderate to dense callosal connections, including the part bordering Area 18. In summary, callosal connections do not appear to be restricted to the region of the 0^{o} vertical meridian, except possibly for Area 17. Each visual area has its own pattern of connections, and similar patterns were noted across primate species.

Supported by NIH Grant EY-02686.

353.8 THE NEURAL DYNAMICS OF BINOCULAR FORM PERCEPTION. S. Grossberg and M.A. Cohen*. Center for Adaptive Systems, Boston University, Boston, MA 02215.

A heterarchical neural network has been derived whose several stages lead to global representation of depth, form, and lightness information. The several stages of network processing may be compared to stages of visual cortical processing. It is shown how monocular preprocessing using tuning, contrast, and matching properties of feedback competitive networks, adaptation and habituation of chemical gates, filtering and learning properties of long-term memory traces, and smoothing properties of nonlinear diffusion among cellular compartments set the stage for eliciting quantized nonlinear standing waves among multiple spatial scales. The resonant ensemble of such waves constitutes a global visual representation that embodies depth, lightness, and form information about a visual scene. The standing wave representation provides a physical interpretation of the Fourier theory of spatial vision in a processing framework that also explicates concepts of adaptive feature extraction and cooperativity.

353.9 TEMPORAL FREQUENCY SELECTIVITY OF HUMAN STRIATE CORTEX DEMONSTRATED BY POSITRON EMISSION TOMOGRAPHY, P.T.Fox* and M.E.Raichle. Department of Neurology and Division of Radiation Sciences, Washington Univ. School of Med., St. Louis, MO 63110.

Regional cerebral blood flow (rCBF) is known to vary in tandem with changes in regional neuronal activity rate (rNAR) and with the regional rate of metabolic substrate uptake. Non-invasive measurements of rCBF require accumulation of radioactive counts over a finite time. The resultant value of rCBF reflects neuronal events throughout the period from isotope injection to scan completion, approximately 50 seconds by our method. Sensory stimulation would be anticipated to increase rNAR in primary sensory cortex in a rate dependent manner with the mean rNAR and rCBF rising as stimulus rate rises. Previous rCBF studies have failed to demonstrate such a relation.

Forty-eight $H_2{}^{15}O$ positron emission tomographic scans were performed in 6 normal volunteers, each subject undergoing 8 scans during a single session. The initial and final scans were unstimulated with eyes and ears occluded. During the remaining 6 scans patterned flash visual stimulation was given via light emitting diode dot grids in goggle eye-pieces (Grass S10VS), a stimulus known to elicit consistent visual evoked potentials (VEPs). Stimulus rate was varied from scan to scan in random order with each subject receiving stimuli at 1.0Hz, 3.9Hz, 7.8Hz, 15.5Hz, 33.1Hz, 60.0Hz. Flash intensity and duration were constant.

Cortex responsive to visual stimulus was located on the mesial and polar occipital surfaces, anatomically demonstrated to be striate cortex. Occipital rCBF percent change from the unstimulated state was calculated for each subject in each test condition for a 27mm by 13.5mm midline region extending anteriorly from the occipital pole. Mean rCBF increase for each condition was as follows: 1.0Hz=8.8%; 3.9Hz=23.4%; 7.8Hz=33.4%; 15.6Hz=28.9%; 33.1Hz=18.6%; 60.0Hz=19.8%. In every subject occipital rCBF varied systematically with stimulus rate. The rCBF response for each stimulus condition is significantly different from unstimulated control ($p<.001$) except 1.0Hz ($p>.1$), by Dunnett's test for multiple comparision. The changes in rCBF associated with changes in stimulus rate are also highly significant ($p<.001$), by single factor ANOV.

Several conclusions may be drawn. 1) Striate cortex rCBF and presumably rNAR vary systematically with photic stimulus rate with a peak response at 8Hz. 2) This rCBF peak at 8Hz corresponds to the psychophysical and electrophysiologic maximum response at alpha frequency known as the Brücke-Bartley effect. 3) Pattern flash, known to elicit scalp recorded VEPs, selectively changes rCBF in striate cortex, corroborating the site of dipole flux in VEPs as striate cortex.

NEURAL PLASTICITY IN ADULT ANIMALS IV

354.1 2-DEOXYGLUCOSE STUDY OF VESTIBULAR COMPENSATION AFTER LABYRINTHECTOMY: CHANGES IN VESTIBULAR AND AUDITORY PATHWAYS, CEREBELLUM, AND EXTRAOCULAR MUSCLE NUCLEI. W.H.M.L. Luyten, F.R. Sharp and A.F. Ryan*. Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093.

Vestibular compensation, an impressive demonstration of (adult) CNS plasticity, has been investigated extensively. Although the 2-deoxyglucose (2DG) method has proven a useful technique for the functional mapping of polysynaptic pathways, we are not aware of its use in the study of vestibular compensation.

Female Sprague Dawley rats weighing between 190 and 240g underwent right labyrinthectomy under ether anesthesia. To make sure no functional end-organ would remain, Gelfoam(R), soaked in concentrated Streptomycin solution was placed into the created cavity. Only subjects that showed evidence of acute vestibular damage (nystagmus, tonic neck and body deviations, ataxia), were kept for further study. At various time intervals (3 1/2 h, 24 h, 7 and 20 d) after the lesion, ^{14}C 2DG was injected by tail vein. The animals were sacrificed 45 min. later, decapitated, and the brain was frozen, sectioned, and autoradiographed.

Quantitative autoradiography showed:

1. Vestibular Nuclei: the asymmetry in metabolism (lower 2DG incorporation on the lesioned side) is greatest initially, and tapers off over the observed period. After 20 days, symmetry has been reestablished in superior and spinal, but not yet in lateral and medial vestibular nuclei.
2. Oculomotor and trochlear nuclei showed large differences (more 2DG incorporation on the lesioned side) that remained essentially unchanged over the 20 day period.
3. Cerebellum: small but consistent differences (less 2DG incorporation on the lesioned side) were seen in the deep cerebellar nuclei, having disappeared in the fastigial nucleus by 20 days, but not in the interstitial or dentate nuclei.
4. Auditory System: large differences, constant over time, were seen throughout auditory pathways: on the lesioned side less 2DG was incorporated into the cochlear nuclei, and on the contralateral side less 2DG was incorporated into lateral lemniscus, inferior colliculus, medial geniculate and auditory cortex.

This study illustrates lack of functional compensation after cochlear damage as opposed to vestibular compensation following labyrinth destruction. It supports the conclusion that vestibular compensation is due to normalization of activity in the vestibular nuclei and suggests that deep cerebellar nuclei may in part be responsible. The changes in VIth and IVth nerve nuclei are not the result of nystagmus (which disappears in 3-4 days) but are interpreted to reflect eye movements compensating for the tonic neck deviation that still remains even after 20 days.

354.2 SHARED CHARACTERISTICS OF POTENTIATION AND DEPRESSION IN THE EC-DG SYSTEM. B. Burger and W. B Levy. Dept. of Neurosurgery, University of Virginia School of Medicine, Charlottesville, VA 22908.

Studies of what is known as long-term potentiation (LTP) carried out at the excitatory entorhinal (EC)-dentate (DG) synapses contribute to knowledge of synaptic modifiability. Such studies adduce the associative nature of LTP, the existence of a process by which LTP can be removed using the very same postsynaptic excitation which permitted the potentiation, and the individual modifiability of individual synapses. In our studies the weak contralateral EC-DG projection is the test system. Brief high frequency stimulation here produces no consistent change of synaptic efficacy. Yet high frequency activation of the test system in conjunction with a powerful, synaptically generated, excitation of the postsynaptic elements produces LTP of the test system. This same activation of strong postsynaptic excitation, if not paired with activity in the test system, reduces the synaptic response in the test system. The original report named this depression depotentiation since it was obtained on a background of experimentally induced potentiation. If this depression is physiologically important then it should occur without the prior LTP. Moreover, such depression should be as long term as LTP itself. Using standard methods the synaptic efficacy of the contralateral EC-DG test system in albino rats is depressed by conditioning the convergent ipsilateral system without prior experimentally induced potentiation. Specifically, a depressed contralateral response follows from conditioning the powerful converging ipsilateral system with 5 sets of four 8 pulse 400Hz trains of stimulation. Trains were delivered 1/10s; 5 min of testing separated sets of trains. Synaptic depression was seen in all 10 animals studied. The 8 with unambiguously quantifiable contralateral waveforms averaged 67% of their initial amplitude at the end of conditioning. Testing 1 hr after the last conditioning train revealed the permanent nature of this depression; there was no detectable change in response amplitude immediately post-conditioning compared with 1 hr later. Since there exists no temporal decay of depression, the depressed synaptic efficacy appears as long-term as the potentiated synaptic efficacy studied in this system. The fact that individual synapses are individually modifiable; that the same events showing the characteristics of a postsynaptic response, permits both depression and depotentiation; that there is no need to posit a passive decay process; and that both potentiation and depression are induced stepwise to asymptotic values allows for a simple algebraic statement of the phenomena of long-term associative potentiation/depression. c.f. McNaughton et al. Brain Res. 157, p. 277; Levy & Steward, ibid. 175, p. 233; Levy et al. Neurosci. 8, p. 799.

354.3 INCREASED TRYPTOPHAN HYDROXYLASE ACTIVITY IN CENTRAL SEROTONERGIC NEURONS SPARED BY 5,7-DIHYDROXYTRYPTAMINE. Michal K. Stachowiak*, Jacob H.Jacoby, Michael J. Zigmond and Edward M. Stricker. Departments of Biological Sciences, Psychiatry and Psychology, University of Pittsburgh, Pittsburgh, PA 15260.

We wished to determine whether compensatory neurochemical changes occurred in residual serotonin (5HT)-containing neurons after partial destruction of a central 5HT pathway. Male rats, 200-250 g, received 5,7-dihydroxytryptamine (5,7-DHT, 120 ug, ivt) 1 hr after treatment with nomifensine (15 mg/kg, i.p.). Three or 21 days later they were killed by decapitation and three brain areas were removed: midbrain, an area rich in 5HT cells, septum, an area containing 5HT terminals, and hippocampus, a more distal terminal field. 5HT levels in these areas were reduced by 55%, 90%, and 90%, respectively, suggesting that the neurotoxin had produced a profound degeneration of 5HT terminals with less impact on 5HT cell bodies. Three days post-lesion, tryptophan hydroxylase (TPH) activity was measured in supernatants prepared from each region. In hippocampus, TPH activity was reduced by only 80%, resulting in a 2-fold increase in the ratio of TPH activity to 5HT. This was associated with a decrease in the Km of TPH for tryptophan from 0.100 mM to 0.051 mM and a 34% increase in the ratio of Vmax to 5HT levels. These effects could be mimicked in vitro by the addition of Ca^{++} (100 uM), Mg^{++} (3 mM) and ATP (.5 mM) to preparations from control tissue, whereas Ca^{++} had no further effect on TPH activity in hippocampus from brain-damaged animals. In contrast, while TPH activity also was increased relative to 5HT content in midbrain and in septum, TPH in these structures could be further stimulated by Ca^{++}. At 21 days post-lesion, TPH activity in hippocampus and septum, but not midbrain, was still elevated relative to 5HT content. Moreover, hippocampal TPH could now be further stimulated in vitro by the addition of Ca^{++}. All increases in TPH activity were accompanied by an increase in 5HIAA/5HT suggesting an increase in 5HT turnover in residual neurons.

These results indicate that after partial injury to 5HT projections, TPH activity increases in the neurons that remain. Initially this may be associated with an activation of existing TPH molecules which resembles that produced in vitro by calcium-dependent phosphorylation. Later the increase in TPH activity instead may be due to accumulation of TPH protein, which can be seen first in areas near 5HT cell bodies and then in more distal terminal regions. These changes are reminiscent of similar changes in tyrosine hydroxylase activity observed in central noradrenergic neurons after 6-hydroxydopamine treatment (Acheson & Zigmond, J. Neurosci: 1, 493, 1981). Such changes in residual neurons after partial injury may contribute to the restoration of synaptic function within the damaged system.

354.4 INCREASED ADRENERGIC PROJECTIONS FROM THE LOCUS COERULEUS TO THE LATERAL GENICULATE NUCLEUS FOLLOWING ABLATION OF THE VISUAL CORTEX IN ADULT RATS. S. Nakamura, T. Sakaguchi* and T. Shirokawa*. Dept. of Neurophysiology, Inst. of Higher Nervous Activity, Osaka Univ. Med. Sch. , Kita-ku, Osaka 530, Japan.

Stenevi et al. showed using histofluorescence techniques that unilateral ablation of the visual cortex (VC) in adult rats resulted in an increase in adrenergic terminals of the lateral geniculate nucleus (LGN) (Exp. Neurol., 35:290, 1972). Present experiments were carried out to confirm their result and then to see if the increased adrenergic terminals in the LGN after VC ablation were due to an increase in projections from the locus coeruleus (LC) to the LGN. The rate of LC neurons activated antidromically from the LGN to the total number of LC neurons recorded was taken as a physiological index for the amount of the projections from the LC to the LGN.

Male and female Sprague-Dawley rats were used for histofluorescent and electrophysiological studies. Unilateral VC was removed at age of 8 to 12 weeks and experiments were made between 2 and 7 weeks after operation. For histofluorescent studies, animals were sacrificed by decapitation and the diencephalon containing the LGN was dissected out and processed for fluorescence microscopy according to the Falck and Hillarp method. For electrophysiological studies, rats were anesthetized with urethane (1.3 g/kg, i.p.) and fixed to a stereotaxic apparatus. Single unit discharges were recorded extracellularly from the LC by means of a glass micropipette filled with 3 M KCl. Stimulating electrodes were placed in the bilateral LGN and the frontal cortex (FC). Pulses applied to these sites were delivered with a pulse width of 0.7 msec and at currents of 0.1-5 mA.

Histofluorescent studies indicated that the number of adrenergic terminals in the LGN ipsilateral to the removed VC was remarkably increased following ablation of the VC. This corresponds well with the result of Stenevi et al. Based on this finding, antidromic stimulation techniques were employed to estimate the amount of the projections from the LC to the LGN. In normals 28% of LC neurons recorded were found to project to the LGN, while the rate of LC neurons projecting to the LGN was increased to 37% after VC ablation. In addition, LC neurons innervating the LGN and the FC simultaneously were more frequently recorded in operated rats than in normals. These findings suggest that many of LC neurons which had projected to the FC as well as the VC before VC ablation resulted in forming axon collaterals to the LGN after removal of the VC (pruning effect), thus contributing to the increased projections from the LC to the LGN.

354.5 INTRACELLULAR AND EXTRACELLULAR RECORDINGS FROM SEPTAL TRANSPLANTS IN VITRO AND IN VIVO. S.R. Thomas*, S.B. Dunnett* and J.S. Kelly. (SPON: European Neuroscience Association). Department of Pharmacology, St. George"s Hospital Medical School, Tooting, London S.W.17 ORE, U.K.

Previously, we reported that neural transplants of embryonic septal nuclei into the brains of adult rats with septo-hippocampal lesions support recovery of synaptic as well as behavioural function: stimulation of the transplant evoked field responses and pronounced heterosynaptic facilitation of perforant path-evoked responses in the dentate gyrus of the host hipppocampus (Low, Lewis, Bunch, Dunnett, Thomas, Iversen, Bjorklund and Stenevi, Nature 300: 260, 1982). However, no data was available on the cellular properties of these transplanted neurones. We have now extended these studies with further extracellular recordings in vivo, and with the first intracellular recordings from grafted septal neurones in a composite transplant-hippocampal slice maintained in vitro. Composite slices were prepared by dissecting the host hippocampus with the transplant attached to its septal pole and cutting oblique slices on a McIlwain tissue chopper at a nominal thickness of 400 m. The slices were then transferred to a tissue chamber and recordings were made using conventional intracellular techniques at 33-35 C. Our preliminary intracellular observations on cells from four successfully implanted rats show transplant neurones in vitro to have normal monophasic action potentials and resting potentials around -50 mV. We shall report further on attempts to characterise transplant neurones and transplant-host interconnections with intracellular recording techniques. The results to date indicate that the composite transplant-hippocampal slice is a viable preparation and that active neurones are present in the transplanted septal tissue.

354.6 ESTROGEN INFLUENCES AXON SPROUTING IN THE HIPPOCAMPAL DENTATE GYRUS. J.K. Morse, S.W. Scheff and S.T. DeKosky. Depts of Anatomy and Neurology, Univ. of Kentucky and V.A. Medical Center, Lexington, KY 40536:

The hippocampal dentate gyrus has been used extensively to study plasticity in the CNS. Following partial deafferentation of the granule cell dendritic tree, a morphological change occurs among the residual afferents innervating the dentate molecular layer. One aspect of this change is the growth of commissural and associational fibers following a lesion of the ipsilateral entorhinal cortex. We have previously reported on the influence of glucocorticoids as they affect the growth of this fiber plexus. The present study was designed to test the influence of estrogen on this same growth response.

Young adult animals of both sexes were employed and were randomly assigned to one of three different groups: A) castration and a 5mm estradiol implant (resulting in a serum concentration of 75 pg/ml), B) castration and a cholesterol implant, and C) sham castration and a cholesterol implant. Seven days following the castration procedure, the animals were implanted and subjected to a unilateral removal of the entorhinal cortex. Following a survival period of 15 days the animals were killed and the brains processed for changes in brain morphology as a result of the entorhinal lesion. The commissural and associational fibers were followed with the Holmes fiber stain and revealed statistically significant differences between groups. At the time of this abstract preparation at least 7 animals have been analyzed for each group. In control animals of both sexes (Group C) a significant sprouting response was observed in agreement with previously reported results. Castrated females without estradiol (Group B), showed a significant decrease in sprouting ($p < .05$) compared to the conttrol animals. Castrated females given estradiol (Group A) showed a sprouting response equivalent to control animals and significantly better ($p < .05$) than castrated females without estradiol (Group B). Male animals showed no effect of either castration alone or castration with estradiol implants compared to male control animals. These results indicate that estrogen may play a role in the sprouting response observed in the female animals.

(Supported by NIH grants NS16981 and NS00444, and the V.A. Medical Research Service.)

354.7 HIPPOCAMPAL SYMPATHETIC INGROWTH IN GUINEA PIG DIFFERS FROM RAT. Genevieve Laforet* and James N. Davis. Veterans Administration Medical Center, Durham, N.C. and Departments of Medicine (Neurology) and Pharmacology, Duke Univ. Med. Ctr., Durham, N.C. 27710.

The adult central nervous system can participate in a variety of neuronal rearrangements in response to injury. One such response occurs in the hippocampal formation after damage to the medial septal nucleus. Work in our laboratory has demonstrated that peripheral noradrenergic fibers originating in the superior cervical ganglion actually grow into the hippocampus in apparent replacement of damaged cholinergic septohippocampal fibers. This sympathetic fiber ingrowth, first described in rat, follows a precise topography within the hippocampus which corresponds the distribution of mossy fiber axons of dentate granule cells. Sprouting fibers are seen above and below the granule cell layer and in the hilus of the dentate as well as in stratum radiatum of area CA_3 of the hippocampal gyrus. No ingrowth is ever seen in stratum pyramidale or area CA_1. Experiments presented here demonstrate that sympathetic ingrowth also occurs into adult guinea pig hippocampus after lesions of the medial septum. Guinea pig hippocampal ingrowth as demonstrated by catecholamine histofluorescence resembles that of the rat in many respects, but differs markedly in its topography. Unlike in the rat, guinea pig ingrowth occurs not only in CA_3 but also in CA_1 with involvement of the pyramidal cell layer as well as stratum oriens and radiatum. This pattern of sympathetic ingrowth in guinea pig does not correspond to the topography of mossy fibers. Neither does it match the distribution of septo-hippocampal projections as demonstrated by anterograde transport of wheat germ agglutinin-coupled horseradish peroxidase from medial septum to hippocampal formation. These studies show that the topography of sympathetic ingrowth after septal injury in the guinea pig differs substantially from the rat and corresponds neither to the distribution of mossy fibers nor to the projections of medial septal afferents to hippocampus. Previous studies have suggested that a target factor perhaps elaborated by the mossy fibers is responsible for initiating sympathetic ingrowth. The disparity between ingrowth pattern in guinea pig and rat suggests that the final topography of ingrowth is regulated differently in these two species. This difference in sympathetic ingrowth between the two species may prove a useful tool for delineating the relative roles of environmental and neuronal properties in determining the final topography of a neuronal rearrangement.

(Supported by NS 06233)

354.8 LESION-INDUCED TRANSNEURONAL PLASTICITY IN THE RAT HIPPOCAMPUS: DEMONSTRATION BY QUANTITATIVE ELECTRON MICROSCOPY. S.F. Hoff. Dept. Pharmacology, The Chicago Medical School, North Chicago, IL 60064.

The process of reactive synaptogenesis has been demonstrated within the denervated zones of the dentate gyrus after a complete unilateral entorhinal lesion. During this process, we have reported that a complete cycle of non-degenerative synapse turnover occurs within the non-denervated ipsilateral inner molecular layer and throughout the contralateral dentate molecular layer (Brain Res. 222: 1-13, 1981). This suggests the potential for additional circuitry remodeling after lesions in areas of the brain that are one or more cells removed from the denervated zones. The potential for transneuronal plasticity has been suggested by several laboratories, and we report here our initial quantitative study of transneuronal plasticity in the CA4/hilus region of the hippocampal formation.

Ninety day old Sprague-Dawley rats received a complete unilateral electrolytic lesion of the entorhinal cortex. The animals were allowed to survive for 2, 4, 10 or 60 days post-lesion and were then sacrificed and prepared for electron microscopy. Over the time course, synaptic profiles were quantified on micrographs taken randomly across the CA4/hilus region, in an area between the midpoints of the dorsal and ventral blades of the dentate granule cell layer. So far 9446 synaptic profiles have been counted over 56,900 square microns of neuropil.

Within the ipsilateral CA4/hilus region we observe a 41% decrease in synaptic density at 4 days post-lesion. The normal synaptic density is 21.3±2.9 synapses/100 square microns and at 4 days post-lesion the density is 12.5±1.8 synapses/100 square microns. By 60 days post-lesion the synaptic density is about 15% below normal values (18.1±2.6 synapses/100 square microns). The mechanism for this change in synaptic density is not clear. We have observed active astrocytes in this area and occassionally a degenerating dendrite which is synapsing with mossy fiber terminals. In addition several degenerating neuronal cell bodies were observed in the hilus region. It may be that a portion of the CA4/hilus neurons are eliminated shortly after an entorhinal lesion because of an altered output from the denervated granule cells, which may account for the sudden decrease in synaptic density. Also, after the dentate molecular layer has been reinnervated, the normal synaptic density may be restored in the hilus as well. Further study is required to clarify these possibilities.

From our initial quantitative data, it appears reasonable to state that transneuronal plasticity does occur and may provide further compensatory mechanisms for the CNS for maintaining homeostasis under various conditions.

354.9 NEOVASCULAR GROWTH THROUGH NONTOXIC BIORESORABLE NERVE GUIDES THAT BRIDGE TRANSECTED ADULT RAT OPTIC NERVE. D. Greatorex*[1], R. Madison[1], R.L. Sidman[1], E. Nyilas*[2], and T.H. Chiu*[2] (SPON: M. Gallagher). Departments of Neuroscience and Neuropathology, Childrens Hospital and Harvard Medical School[1], and Instrumentation Laboratories, Inc.[2], Boston, Ma. 02115.

We have recently been sucessful in using nontoxic bioresorable "nerve guides" to bridge the intracranially transected optic nerve of adult rats (see Madison, et. al. this volume). This study reports the appearance and amount of vascular growth through such implanted neural prostheses at various times after surgery. The guides are fabricated as polymers of synthetic L-polylactates, and are observed to be completely nontoxic and bioresorbable.

A 1.5 mm length of the guide (0.75mm I.D., 1.10mm O.D.) was filled with a collagen matrix (bovine; 95% Type 1, 5% Type 3; Vitrogen, Flow Laboratories) containing 0.5 mg/ml fibrinogen (bovine, Cal Biochem) and 0.4 mg/ml fibronectin (bovine, Kor Biochemicals), and was then implanted with both distal and proximal nerve stumps inserted into the guide. Animals were killed 4 (N=1), 6 (N=2), and 12 (N=2) weeks following surgery. Following perfusion, the nerve guide was dissected free, embedded as one piece in plastic and 1μ sections were cut in cross section and stained with toludine blue.

Data were collected with a computer-controlled light microscope at a final magnification of 1600X. Blood vessels were identified and their lumenal area entered on-line from a digitizing tablet to a display terminal and then to a VAX-11/780 computer for further analysis. Data were collected from 3 points within the nerve guide; proximal, middle, and distal.

The absolute number of blood vessels increases dramatically from the 4 week time period (mean of 6.6) to the later time points (mean of 22). By contrast, the ratio of blood vessel volume to total volume of the tissue cable in the nerve guide decreases over time; 9.4% at 4 weeks, 7.0 % at 6 weeks, and 4.1% at 12 weeks. This decreasing percentage is due to an overall increase in nonvascular elements in the tissue cable since the mean blood vessel area remains relatively constant over all the time points.

These data demonstrate several important points. First of all, the ability of the nerve guide to support vascular growth in the adult mammalian CNS is clearly shown. Secondly, it would appear that vascular elements make up a major component of the early tissue growth through the guide. This vascular foundation may serve not only as a source of nutrition but also as a proper substrate for nonvascular growth (including axonal) which increases over time. Supported by NIH grants NS14768, NO07017, and HD06276.

354.10 HYPOTHALAMIC 5HT AND SEXUAL BEHAVIOR: CORRELATIONS AFTER INTRA-HYPOTHALAMIC 5,7-DHT AND FETAL RAPHE TRANSPLANTS. V.N. Luine, K.J. Renner*, M. Frankfurt, and E.C. Azmitia. The Rockefeller University, New York, NY and Mt. Sinai School of Medicine, New York, NY.

Stereotaxic placement of 5,7-dihydroxytryptamine (5,7-DHT) into the basomedial hypothalamus results in selective loss of 5HT containing neurons and a facilitation of female sexual behavior (lordosis) approximately 10 days later (Brain Res. 264:344, 1983). Facilitated behavior is maintained for 40 days post lesion; thereafter, facilitation gradually declines and has disappeared approximately 55 days after the lesion. Loss of facilitated behavior may be associated with re-innervation of the hypothalamus by 5HT. In some females, 5HT containing fibers and sprouting fibers can be visualized in the hypothalamus immunocytochemically using the immunoperoxidase technique. During the period of facilitated behavior, supersensitivity of 5HT receptors was demonstrated using the 5HT agonist, α-methyl tryptamine (α-MT). In normal hormone primed females, α-MT inhibits lordosis. In 5,7-DHT treated females, α-MT administration abolished lordosis at a dose which was ineffective in normal females. Behavioral supersensitivity was shown at 28 but not 21 days after the lesion.

The ability of raphe cell transplants to more rapidly reverse the facilitatory effect of 5,7-DHT was also investigated. One week after 5,7-DHT placement, a suspension of raphe tissue obtained from 17 day gestation rats was stereotaxically placed in the basomedial hypothalamus of female rats. Experiments thus far indicate that transplants near the ventromedial nucleus rapidly reverse the facilitation of behavior by 5,7-DHT. Implants dorsal or anterior to the ventromedial nucleus are less effective. 5HT was measured immunocytochemically or chemically (HPLC with electrochemical detection) in raphe implants, raphe nuclei and preoptic-hypothalamic nuclei. The presence of implants in lesioned females led to higher levels of 5HT in some nuclei.

These results lend further support to the hypothesis that serotonergic innervation of the ventromedial nucleus exerts tonic inhibition on the induction by gonadal hormones of female sexual behavior. They also suggest that fetal raphe transplants into the hypothalamus may exert functional regulation on induction of behavior. (Supported by USPHS Grant HD12011 and NSF Grant BN579-064-74 and a Hirschl Career Award to ECA).

354.11 A LIGHT AND ELECTRON MICROSCOPIC IMMUNOCYTOCHEMICAL STUDY OF TRANSPLANTED FETAL NEURONS IN ADULT RAT HIPPOCAMPUS AND THE ONSET AND DURATION OF THE ASTROCYTIC RESPONSE. P.J.Gannon*, P.M.Whitaker and E.C.Azmitia. (SPON: M.VanWoert), Mt.Sinai School of Med., New York, N.Y. 10029. An astrocytic glial scar is formed in response to the microinjection of fetal raphe tissue into adult rat hippocampus. (Azmitia E.C.,Whitaker P.M. Neurosci Lett. in press) This study analyses in greater detail the earlier time points after transplantation (TP), using both light and electron microscope immunocytochemical visualisation of the glial reaction and the ongoing viability of the serotonergic neurons. The midbrain raphe area was dissected from 14-15day rat fetuses. The tissue was minced and unilaterally injected with a glass micropipette (tip diameter 250u) into the adult rat (male Sprague Dawley, 220g) hippocampus using stereotaxic coordinates; Anterior 4.5mm, lateral 1.5mm, vertical 3.7 to 4.2mm (relative to the lambda suture). Transplant volumes of 1.5-2.0ul were injected over an area extending 0.5mm, at an injection rate of 0.2ul/min. Specific antibodies were used against Glial Fibrillary Acidic protein (GFA) 1:1500 (Cedarlane Laboratories Ltd.) for astrocytes and serotonin (5HT) 1:1500 (gift of Dr.J.Lauder) for the serotonergic raphe neurons. Fixation, sectioning and immunocytochemical procedures (after primary antibodies) have been described previously (Azmitia E.C., Gannon P.J. Neurosci Abst.38.3, 1982.) At 4 days post TP a dense border of GFA positive staining was seen completely surrounding the transplant, up along the injection tract and extending to a lesser degree throughout the ipsilateral hippocampus. The 5HT-IR cell bodies were distributed throughout the transplant. EM studies showed densely labelled 5HT and GFA-IR cells. Processes were identified both within and outside the transplant area. At later times (13,20,26,days and 2 1/2 and 6months) post TP the GFA-IR cells were still present. At the EM level they were seen to contain 'inclusion bodies' containing vesicles and were quite commonly seen to be adjacent to or encircling endothelial cells. At the later time points 5HT IR cells were seen mainly at or close to the border with the host tissue. Some cells in rare instances even appeared to have migrated into the host tissue. The inner regions of the transplant were usually devoid of 5HT IR cells at the later time points. EM studies showed contacts between 5HT IR processes and non-5HT IR cells both within and outside the transplant. This study, by extending our previous observations to the ultrastructural level provides additional information on the role and function of the adult CNS gliotic response. Supported by NSF grant BNS79-06474

354.12 MARKED PLASTICITY IN CONTACT AREA OF PRESYNAPTIC SPECIALIZATIONS. S. Chen and D.E. Hillman. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016

Plasticity in the number of synaptic contacts has received much attention in the last decade with demonstration that reinnervation of targets by remaining afferent fibers reached as high as 50 to 60%. Quantitative data following graded levels of reduction in the number of parallel fiber afferents to Purkinje cells now reveals that contact area of presynaptic specializations increased by 200% to cover postsynaptic sites before vacant sites began to appear. This modification in axons is indicative of an even greater potential for compensation by presynaptic specializations without increases in the axonic arbor.

Parallel fiber afferents to Purkinje cells of adult rats were lesioned by undercutting the molecular layer. At 7 days to 3 weeks postsurgery, the animals were sacrificed and the molecular layer overlying the lesion was prepared for thick and thin sectioning. Synaptic size and number were quantitated for comparisons over a range of reductions in afferents to 100%. Total synaptic number on each neuron was determined from measurements of volume density and estimates of molecular layer volume obtained from measurements of layer thickness. Stereological techniques were used for density of synapses. The average length of profiles of specialization was determined from large samples recorded by an automated computerized-EM technique and then was converted to contact area by considering the synapse as a disk. Comparison of the reduction in the number of PSDs on each Purkinje cell and the average size of the contact area of sites revealed a reciprocal between the number and area of contact specializations for reductions up to 65%. At reductions greater than this, vacant postsynaptic specializations began to appear but were only half the size of controls and were doubled in number. Quantitation showed that the total presynaptic contact area had increased by 3-fold at reductions of 65% in order to cover the postsynaptic specializations. Although new synaptic formations can be found, there was a preference for enlargement of contact sites of remaining synapses. This 200% increase in the total area of presynaptic specializations contrasts markedly with a constancy in total contact area of the postsynaptic specializations (Neuroscience 6: 1263-1275, 1981). Research supported by USPHS Grants HD-10934 and NS-13742.

354.13 AMPHETAMINE PRODUCES AN ENDURING RESTORATION OF BINOCULAR DEPTH PERCEPTION IN CATS AFTER BILATERAL VISUAL CORTEX ABLATION ONLY IF VISUAL EXPERIENCE IS GIVEN DURING INTOXICATION. Dennis M. Feeney, Ken R. Brock* and David A. Hovda, Depts. of Psychology and Physiology, Univ. of New Mexico, Albuquerque, NM, 87131.

After unilateral motor cortex injury an accelerated recovery of locomotor ability in rats and cats is produced by a single dose of d- amphetamine (AMP) provided that animals are given locomotor experience during the period of drug intoxication (Science, 1982, 217, 855-857; Neurosc. Abst., 1982, 8, 358). However, with 4 doses of AMP in the cat, task specific locomotor experience during intoxication was unnecessary to accelerate recovery of beam-walking. But it is difficult to deprive an animal of locomotor experience. To further examine the importance of experience in AMP-accelerated recovery we studied the visual system where experience is easily controlled. Visual cliff performance was studied in cats with bilateral ablations of areas 17 and 18 to determine the effect of AMP with or without visual experience during drug intoxication. Presurgery tests indicated perfect performance. After injury, predrug baseline tests on postoperative days 4, 6, and 8 indicated all animals were at chance performance. The animals were randomly assigned to one of three conditions: 1) AMP (5 mg/kg,ip) administered on days 10, 14, 18, and 22 postinjury with visual cliff testing at 1, 2, 3, and 6h after drug injection. 2) AMP administered as in condition 1, however, the animals were housed in total darkness for 24h postdrug and given AMP on days 10, 14, 18, 22, 26, 30, and 45 postsurgery. 3) Saline control animals were tested as condition 1. Testing was conducted every other day for 30 days and on days 35, 40, 45, and 50 postinjury. After the first dose of AMP, animals with visual experience during drug intoxication showed a dramatic improvement in performance as early as 6 h postinjection. Neither the saline nor the AMP animals who were housed in the dark during drug intoxication showed any improvement in performance. At 60 days postsurgery animals given AMP and visual experience were completely recovered whereas the other animals showed no sign of recovery. AMP-recovered and normal uninjured animals were then habituated to an eye patch and tested on the visual cliff task using only monocular cues. The perfect level of binocular performance of the AMP-recovered and the normal uninjured animals dropped to chance levels with monocular viewing. We conclude that 4 doses of AMP combined with visual experience during intoxication produces an enduring restoration of binocular depth perception in cats with bilateral visual cortex ablations which if untreated would not recover. Additionally, it is evident that areas 17 and 18 are not essential for stereopsis in the cat. Supported by a grant from the Pennwalt Pharm. Corp.

354.14 SOLEUS MOTOR UNITS IN ADULT SPINAL CATS T.C.Cope M.Fournier, S.C.Bodine, and V.R.Edgerton Dept. of Kinesiology and Brain Research Institute, UCLA, Los Angeles, CA. 90024

Electromechanical properties of single soleus motor units were examined about 4 months after spinal transection at T13 in adult cats (X). In order to identify transection-induced changes in motoneurons and their muscle units, these data were compared with soleus motor units in normal adults (N) since they exhibit relatively homogeneous properties.

	CV(ms)	AHP(ms)	I(nA)	R(MOhms)	CT(ms)	FI	logP/MW
N	70±.8 (86)	142±5.8 (52)	2.6±.2 (50)	2.8±.1 (33)	91±1.7 (110)	.93±.01 (45)	.74±.02 (63)
X	74±.9 *(109)	98±3.9 *(48)	4.6±.3 *(41)	2.4±.1 (34)	63±1.0 *(114)	.91±.01 (37)	.56±.02 *(88)

(mean±sem; *p<0.01)

With regard to motoneuron properties we confirmed previous reports of an increase in axonal conduction velocity (CV) and decrease in afterhyperpolarization duration (AHP) following spinal transection. In addition we found an increase in rheobasic current (I). This change may be contributed to by a slight, albeit insignificant, decrease in input resistance (R). An increase in the rheobasic voltage (IxR) of motoneurons in transected cats suggests a decrease in their intrinsic excitability.

Measurement of isometric contractile properties of single muscle units revealed a decrease in twitch contraction time (CT) and no change in fatigue index (FI). A novel finding was the appearance of sag in records of unfused tension (23 of 75 units) at 10 pps in transected cats. Additionally we found that tetanic tension at 100 pps stimulation frequency (P) dropped after transection even when corrected for their lower muscle weight (MW). Furthermore there was a unique tendency for muscle units with the lowest logP/MW to have the briefest CT.

A noteworthy finding for relationships between motoneurons and their muscle units was a significant positive correlation between CT and AHP ($r=0.71$; $p<0.001$) in transected cats. The slope of this regression line did not differ significantly from normal. This suggests that matching between these properties for single units is either maintained or restored after transection. Supported by NIH Grant 16333

355.1 MUSCLE-LOAD RESONANCE OSCILLATIONS AND MUSCLE ELASTICITY. R. Stiles and H. Nguyen*. Dept. Physiology, Univ. of TN Ctr. Hlth. Sci., Memphis, Tennessee 38163

Little attempt has been made from studies of tremor of a neurally-isolated, muscle-load system to identify the lightly-damped elastic element of muscle that acts with load inertia to determine the frequency of these resonant oscillations. Results from studies on muscle mechanics suggest that this lightly-damped stiffness is due to the short-range stiffness and classical series-elastic elements. In this study, muscle stiffness was computed from values of equivalent load inertia and resonant frequency of a muscle-load system and compared with the stiffness obtained as the slope of whole-muscle length-tension (tension-extension) plots. Resonant stiffness (K_{ω_n}) and length-tension stiffness (K_{LT}) values were obtained for a gastrocnemius-plantaris (GP) muscle pair for each of ten adult Sprague-Dawley rats at two steady-state levels of muscle shortening. For each anesthetized rat (35 mg/kg pentobarbital), the femur of one leg was stabilized, the sciatic nerve cut, and the dissected GP muscles (with intact blood supply) clamped at the Achilles tendon to a pre-loaded lever (Rietz and Stiles, J. Appl. Physiol. 37: 852-860, 1974). Each GP muscle was stimulated to shorten to the steady state length L_1 or L_2 by setting the amplitude of the applied rectangular electrical pulses (1 ms and 28 pps). Damped resonant muscle-load oscillations that occurred in response to mechanical taps applied to the lever were detected at lengths L_1 and L_2 by an accelerometer. The damped resonant frequency of these oscillations was determined by spectral analysis. Tension-extension values were obtained by recording the amount of muscle stretch for after-loads between 0.14 and 0.41 N. For these loads, maximum stretch was less than 2.0 mm, and the tension-extension plots were highly linear.

Results show: 1) K_{ω_n} is about twice the value of K_{LT} when these values are determined at the same muscle length. 2) K_{ω_n} and K_{LT} increase approximately in parallel with muscle shortening. 3) The mean of the intercepts of the zero added weight line by the $K_{LT}(L_1)$ or $K_{LT}(L_2)$ regression lines was not significantly different from mean L_1 or L_2, respectively. We conclude: 1) K_{ω_n} and K_{LT} represent separate active stiffness elements, i.e., no evidence for yielding was obtained. 2) K_{ω_n} likely results from the in-series, lightly-damped short range and series elastic elements of muscle. Supported by USPHS Grant NS 14730 and NIAMDD Grant NRSA T35-AM07405.

355.2 LIMITATIONS OF THE SPIKE TRIGGERED AVERAGING TECHNIQUE FOR OBTAINING SINGLE MOTOR UNIT TWITCH PROFILES. Parveen Bawa and B. Calancie. Dept. of Kinesiology, Simon Fraser University, Burnaby, B.C. V5A 1S6, Canada.

The spike triggered averaging (STA) technique, first introduced to average EPSPs in spinal motoneurons (Mendell and Henneman, 1971, J. Neurophysiol. 34: 171-187) was applied by Stein et al. (1972, Brain Res. 40: 187-192) to average motor unit twitch characteristics in humans. The technique requires the subject to isometrically activate a single motor unit (SMU) at it's minimal tonic firing rate. Typical firing rates for motor units in upper limb muscles (those containing both slow and fast units) vary between 7-12 pps. To what degree, if any, do these firing rates alter the observed twitch profile of a unit when compared to it's true, single twitch profile?

We tested the effect of stimulation at different rates on the twitch profile obtained from cat SMUs. The mean interspike intervals for stimulating pulses either (a) had no variability (regular rate), or (b) varied about a mean value (irregular rate). Irregular rates were obtained from records of human motor unit activity, and were used to drive a stimulator at different rates. Single units in soleus and medial gastrocnemius were obtained from teased ventral root filaments and placed on platinum hook electrodes for stimulating. All units studied showed a monotonic decrease in twitch tension amplitude, contraction time, and half relaxation time as the stimulus rate increased from .5 to 10 pps. The percentage decrease in these three parameters was not the same. Twitch amplitude decreased more than the contraction time and the half relaxation time, thus producing a nonlinear change in the twitch profile. Twitches with short contraction times were affected less than the ones with longer contraction times over the rates of stimulation used. Comparison of regular vs. irregular rates of stimulation on the tension profile of a unit showed no consistent differences in measured parameters.

Although spike triggered averaging is a useful technique to study the recruitment pattern of single motor units during voluntary activation, the absolute values of various mechanical parameters as measured are at best only approximations of that unit's true mechanical properties.

Supported by NSERC grant. B. Calancie is supported by NSERC studentship.

355.3 MECHANICAL PROPERTIES OF TROCHLEAR-SUPERIOR OBLIQUE MOTOR UNITS IN THE CAT. J.S. Nelson*, S.J. Goldberg and J.R. McClung* (SPON: J.A. Astruc). Dept. of Anat., Med. Coll. of Va.-VCU, Richmond, VA 23298.

Motor units of skeletal muscles have been classified into fast fatiguable (FF), fast-fatigue intermediate (FI), fast-fatigue resistant (FR) and slow-fatigue resistant (S) types. Studies of extraocular muscles (EOMs) however, have not systematically examined similarities or differences between EOM motor units and skeletal motor unit types.

The present study was designed to examine the twitch motor units of the cat superior oblique muscle. The twitch motor units (as opposed to non-twitch units) of EOMs are generally thought to be similar to, though faster than, skeletal motor units.

Stimulation of the IVth nerve in the orbit was used to antidromically identify trochlear motoneurons (MNs). The superior oblique tendon was freed from the globe and attached to a force transducer. Motor unit mechanical properties were measured in response to intracellular stimulation. Tetanic stimulation at 150 Hz at a rate of one 500 msec. train per second for a period of two minutes was delivered in order to study motor unit fatigue. The fatigue index of motor units was the ratio of the peak force of the final tetanus to the initial tetanus. MN axonal conduction velocity was also studied in relation to motor unit mechanical properties.

Our preliminary results (based on 14 fully studied motor units plus 12 units in which fatigue could not be tested) show some interesting similarities to skeletal motor unit types. The slowest motor units identified had twitch time and fusion frequency ranges of 11-21 msec. and 90-110 Hz respectively. These slow twitch units were weak, innervated by slow conducting axons and fatigue resistant. A population of faster motor units was identified to have twitch time and fusion frequency ranges of 4.4-9.4 msec. and 150-300 Hz respectively. The faster twitch units showed greater variability in speed, tension production and axonal conduction velocity relationships than did the slower units. Eleven of the 14 fully studied units were fast. Two of the 11 had fatigue indexes of 0.20 or less while 7 had fatigue indexes ranging from 0.37-0.6 and two were fatigue resistant at 0.80. These preliminary results, with the present fatigue parameters, suggest that superior oblique motor units can be classified on the basis of contraction time and fatigue properties.

(Supported by Jeffress Research Grant J-4 and VCU Grants-in-Aid).

355.4 **CONDUCTION VELOCITY IN HUMAN MUSCLES ESTIMATED FROM THE MYOELECTRIC SIGNAL.** **G. Bilotto, H. Broman* and C. J. De Luca, NeuroMuscular Res. Lab., Dept. of Orthopaedic Surgery, Children's Hosp. Med. Ctr., Harvard Medical School, Boston, MA 02115; and Liberty Mutual Res. Ctr., Hopkinton, MA 01748**

The power spectral density of the myoelectric signal from an isometrically contracting muscle is explicitly dependent on the muscle fiber conduction velocity according to a theoretical model (Lindstrom and Magnusson, Proc. IEEE, 765(5): 653-666, 1977). We attempted to directly measure the average conduction velocity by detecting the surface myoelectric signal from the anterior tibialis muscle during constant force isometric contractions. Our efforts were directed at elucidating the following relationships: 1) muscle fiber conduction velocity vs. muscle force output or vs. duration of muscle contraction, 2) changes in spectral parameters as they track the power spectral shift which occur during localized muscle fatigue vs. changes in conduction velocity.

Muscle fiber conduction velocity can be estimated by cross-correlating two surface waveforms obtained from different locations along the muscle fibers. An analog device, using this principle and employing band-pass filtering (80-160 Hz), measured and displayed the average conduction velocity on-line and in real-time. However, conduction velocity estimates were not consistent and in fact, in some subjects tested, the actual values were extraordinarily large. A subsequent consideration of the non-homogeneity and anisotropic properties of muscle and the detecting arrangement led to a re-design of the detecting electrode and recording arrangement. As a result, the same cross-correlation technique yielded reliable and repeatable measurements.

In all eight subjects tested, the conduction velocity measured in non-fatiguing contractions was dependent on the output force: The greater the force, the greater the conduction velocity recorded. Furthermore, the conduction velocity consistently decreased in the anterior tibialis muscle during localized muscle fatigue, as tested at 80% of the maximal force. Preliminary results indicates that also the biceps brachii muscle exhibits this same behavior. These results are in contrast to those of a recent report (M. Naeije and H. Zorn, Eur. J. Appl. Physiol. 50: 23-33, 1982) which indicated that a compression of the power spectral density function during muscular fatigue may occur independently of muscle fiber conduction velocity.

Simultaneous monitoring of various spectral parameters and conduction velocity revealed their relative dependency or their concomitant variations.

(Supported by Liberty Mutual Insurance Company, and the Swedish Work Environment Fund)

355.5 DOES MOTOR UNIT TYPE DETERMINE RECRUITMENT ORDER? Paul Ruenzel*, Parveen Bawa, Marc D. Binder and Elwood Henneman. Dept. of Physiology and Biophysics, Harvard Medical School, Boston, MA 02115.

In a recent paper (Neurosurg. 8: 608, 1981) Munson and Sypert concluded that "sequential recruitment of motor units seems to be based on motor unit type" rather than size. Since the conduction velocities (CVs) of different types of motor units overlap extensively in pooled data, recruitment by type should activate some units in reversed order to that of their CVs. Furthermore, recruitment primarily by type implies reduced control of this orderly process in a homogeneous muscle. To test these possibilities, we conducted a pair-wise comparison of recruitment order and CV in more than 200 motor units of the cats soleus (SOL), which consists exclusively of one type of muscle fiber. Similar comparisons were made in a smaller sample of units in the adjacent heterogeneous muscle, medial gastrocnemius (MG). Recruitment order was determined by recording muscle unit action potentials (MUAPs) during stretch of the triceps surae in decerebrate cats. Axonal conduction times were obtained by using the MUAPs to do spike-triggered back-averaging from the ventral root and muscle nerve. With the 20 μs resolution of this technique, we could confidently rank-order any pair of motor units whose axonal CVs differed by 2 m/s. The sample of CVs in this study ranged from 43 to 77 m/s in SOL and from 73 to 99 m/s in MG.

The order of recruitment was the same as the rank-order of axonal conduction velocities in 89 of 92 pairs in SOL and in 37 of 38 pairs in MG. Only about 3% of units were not recruited in order of their axonal CVs. The statistics were apparently unaltered by the absence or presence of different types of motor units in the two samples, suggesting that recruitment is not significantly influenced by motor unit type. They also indicate that recruitment is not random within a population of units of similar type. The results reveal that the recruitment thresholds of motoneurons in a stretch reflex are highly correlated with the conduction velocities of their axons.

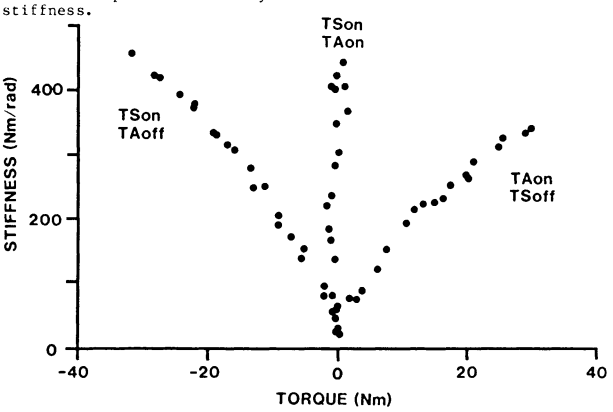
This research was supported by grants from the National Institutes of Health (NS 10857) and from the National Multiple Sclerosis Society (RG 1422-A-1). M.D.B. was supported by a NINCDS Teacher-Investigator Development Award (NS 00345) and by a NSF Grant (BNS82-06223).

355.6 VARIATION OF ANKLE STIFFNESS WITH CO-CONTRACTION. I.W. Hunter, R.E. Kearney and P.L. Weiss. Biomedical Engineering Unit, Faculty of Medicine, McGill University, Montreal, Canada, H3G 1Y6.

Providing co-contraction is avoided there is a linear relation between human ankle stiffness and torque (Hunter and Kearney, J. Biomech., 1982). Purely mechanical considerations (agonist/antagonist stiffnesses add and torques subtract) imply that the stiffness vs torque relation can be altered by co-contraction. Moreover the relation will be restricted to a region in the stiffness/torque plane bounded by a parallelogram.

In the present study of tibialis anterior (TA) and triceps surae (TS) co-contraction we sought to determine the extent to which the nervous system might further restrict the stiffness/force region, and to elucidate the inter-relations between stiffness, torque, and TA and TS mean rectified EMGs.

Subjects were exposed to repeated stochastic ankle angular displacements during contractions of TA and/or TS. System identification techniques were used to determine the non-parametric linear dynamic relation between ankle position and torque. A second-order model was fitted to this relation using parameter estimation procedures to yield an estimate of static ankle stiffness.



The ability of subjects to generate various torque/stiffness combinations is demonstrated in the above figure. The stiffness attained during various levels of co-contraction could be accurately predicted from a simple three parameter non-linear model involving the TA and TS EMGs. The model can in turn be used to separate total torque into agonist and antagonist contributions and thus provide an objective measure of level of co-contraction.

Supported by a grant from the Canadian MRC.

355.7 ANATOMY OF MOTOR UNITS IN THE CAT FLEXOR CARPI RADIALIS MUSCLE. B.R. Botterman, G.A. Iwamoto and W.J. Gonyea*. Dept. of Cell Biology, The Univ. of Texas Hlth. Sci. Ctr., Dallas, TX 75235

The anatomical distribution and histochemical profiles of muscle fibers belonging to single motor units (MUs) were studied in the flexor carpi radialis (FCR) muscle, one of two primary wrist flexors. The FCR is a histochemically "compartmentalized" multipennate muscle, containing an "oxidative" region with a high concentration of SO and FOG muscle fibers, an intermediate region containing an even mixture of the three fiber types, and a "glycolytic" region where FG fibers are more prevalent. Using the glycogen depletion technique, muscle fibers innervated by a MU were identified in frozen sections by the loss of stored glycogen after prolonged stimulation (30 to 90 min) of functionally isolated motor axons. Six MUs were classified on the basis of their mechanical properties into type S, FR, F(int) and FF units. As for other muscles of the cat, there was a correspondence of the three main motor-unit types (S, FR and FF) with the three fiber types (SO, FOG and FG). The one F(int) unit (fatigue index of 0.32) studied was found to innervate fibers which corresponded to a histochemical profile of FG fibers. The muscle fibers of fast-twitch units were found to be scattered throughout an extensive territory, and were not confined to the distinctive histochemical regions of FCR. Previous work has shown that fiber area for FG fibers depends on their location in the muscle, with the largest fibers found in the "glycolytic" region and the smallest in the "oxidative" region. This trend was also observed for fibers of the one FF unit studied, in which mean fiber area for these two regions differed by a factor of 2. In addition, fast-twtich units demonstrated numerous instances of contiguous pairs of depleted fibers, as well as cases of 3 and 4 contiguous fibers. In contrast, the territorial volumes of the two type S units studied were limited to the "oxidative" region of the muscle. Supported by NIH grants NS 17683 to B.R.B. and AM 17615 to W.J.G.

355.8 CONTRACTILE PROPERTIES OF THE NORMAL HUMAN DIAPHRAGM. F. Bellemare*, B. Bigland-Ritchie, L. Delhez*, A. DeTroyer* and J.J. Woods*. Malvoz Inst., Liege, Belgium, and John B. Pierce Foundation, New Haven, CT. 06519.

The mechanical properties of the diaphragm have been evaluated by stimulating the phrenic nerves both unilaterally and bilaterally, and recording the transdiaphragmatic pressure (Pdi) with closed glottis in six normal seated subjects. Diaphragm shortening was minimized by strapping the abdomen and lower rib cage at end expiratory lung volume. Bilateral single maximal shocks elicited Pdi swings (twitches) of 43.3 ± 13.4 cm H_2O or 20.2 ± 4% of the maximal voluntary Pdi (Pdi max: 219 ± 34 cm H_2O). These were 32 ± 3% greater than the sum of the Pdi swings obtained during unilateral stimulation. The shape of single twitches is not regular, but shows two distinct peaks at 61.7 ± 10.6 ms and 89 ± 11.4 ms. measured from twitch onset. These may reflect the mixed fiber type composition of the diaphragm. The Pdi/frequency relationship during bilateral stimulation was determined in two subjects. The highest Pdi was recorded at 35 Hz and appeared totally fused. They corresponded to 82% of the Pdi max. measured during voluntary contractions. The twitch/35 Hz Pdi ratio was 25%. Half the tetanic Pdi was recorded at 15 Hz. When single maximal shocks were superimposed on graded voluntary contractions, the resulting Pdi increment was found to decline as a function of the voluntary Pdi. After several training sessions, no detectable increase in Pdi occured when the subjects generated Pdi max., indicating that their diaphragm was fully activated by voluntary effort. Single twitches were recorded before and after 4s. voluntary contractions sustained at predetermined fractions of Pdi max. (range: 0 to 100% Pdi max.). Twitch potentiation occured following contractions made at Pdi levels greater than 20% Pdi max., and increased as a function of % Pdi max. The maximal potentiated twitches (after contractions at 100% Pdi max) were 37% greater than control values.

It is concluded:1) The contractile properties of the human diaphragm can only be estimated during bilateral stimulation; 2) its contractile properties lie between those of individual human fast and slow twitch muscle fibers, being in fact a reflection of both; 3) the twitch/tetanus ratio during bilateral stimulation is unusually high (about 25%) suggesting a low compliance of the series elastic elements; 4) twitch Pdi is potentiated by up to 37% following voluntary contractions exceeding 20% Pdi max.; 5) the degree of activation of the diaphragm can be estimated by measuring the Pdi increment resulting from single maximal shocks superimposed on a voluntary contraction; 6) normal subjects are capable of fully activating their diaphragm during maximal effort.

Supported by MDA and USPHS grant HL30026

355.9 THE AFTEREFFECTS OF STRETCH AND RELEASE IN CAT SOLEUS MUSCLE. N.C. Kiesler* and T.R. Nichols (SPON: R.S. Hutton). Dept. of Kinesiology, Univ. of Washington, Seattle, WA 98195

Following stretch of activated cat solei, the force remains above the corresponding isometric force level by an amount called the force remainder. Similarly, following a release, the force remains below the isometric level, the difference being termed the force deficit. This experiment was designed to investigate the possibility that force remainder and force deficit are the result of a common mechanism.

Cats were anesthetized with Nembutal and the solei were attached via a force transducer to the pulley of a servo-controlled torque motor. Muscle temperature was maintained at 36°C. Stimulation was applied through the muscle nerve at 20 pps at intervals separated by 1 minute rest periods. Changes in length were applied to the muscle 1.5 seconds after initiation of contraction. Force remainder and deficit were found to be approximately 5 % of the corresponding isometric force following a 15 % change in length. Force remainder and deficit were present even at the slowest velocity of .004 length/s, and they changed less than 60 % over a 200-fold increase in velocity. Force remainder lasted in excess of 1 minute after termination of the stretch and was abolished only by complete relaxation of the muscle. A straight line relationship between force remainder and amplitude of the stretch was found. An increase in temperature led to an increase in both force remainder and deficit. When the onset of the length change was delayed to 5 seconds after the initiation of the contraction, force remainder and deficit were unchanged in magnitude, suggesting that they are not related to the phenomenon of creep (Julian and Morgan, 1979).

Our findings which indicate that force remainder and force deficit vary in similar fashion with variations in velocity and temperature provide evidence for the existence of a common mechanism.

(Supported by the N.I.H. #NS17025 and by the Graduate School Research Fund of the University of Washington)

355.10 EFFECTS OF DOMINANCE, SEX AND PREFERENCE ON PEAK TORQUE VALUES OF UPPER AND LOWER EXTREMITY MUSCLES IN NORMAL YOUNG ADULTS. M. T. Moffroid and S. B. Gutwin*. Department of Physical Therapy, University of Vermont, Burlington VT 05405.

Whereas hand grip strength testing has not demonstrated significant differences between dominant and non-dominant sides (Schmidt, R. and Toews, J., Arch Phys Med 51:6, 1970), results of dynamic testing to assess the effect of dominance on muscle torque have not produced consistent results.

We decided to study torque differences between the dominant and non-dominant sides, and to determine if there was any interaction with preference and sex. Preference was defined as the preferred side, irrespective of dominance.

Using one standard measure of dominance, The Edinburgh Inventory (Oldfield, R. C., Neuropsychologia, 9:1, 1971), 40 randomly selected male and female students were tested for upper extremity dominance and for lower extremity dominance. This assessment identified 35 subjects as right side dominant in the upper extremity (20 were males), and 32 as right side dominant in the lower extremity (16 were males). All subjects were then given the opportunity to become familiar with an isokinetic device which is an electromechanical dynamometer used to quantify muscular torque during dynamic concentric contractions. (Hislop, H. J. and Perrine, J. J., Phys Ther 47:2, 1967). The subjects were then tested for the peak torque of maximal efforts of flexion and extension motions of elbows, wrists, knees and ankles bilaterally at a speed of 1.05 rad/sec. The maximal peak torque outputs were analyzed to determine the relationships of dominance, left and right preference and sex to torque production.

Analysis of variance with repeated measures supported the following statements: 1) no statistically significant differences in torque production between dominant and non-dominant sides in the individual muscle groups tested; 2) a significantly greater torque capability in the right upper extremity in both right and left preferenced individuals; and 3) a significantly greater torque capability in the left lower extremity for all subjects except the left preferenced females.

355.11 THE MOTONEURON POOL OF TRANSPLANTED MURINE MUSCLE, K. Klueber* and M. Ontell. Dept. of Anatomy and Cell Biology, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

Subsequent to whole muscle transplantation, the myofibers of the denervated, devascularized graft undergo necrosis, and the necrotic myofibers are phagocytosed leaving only basal lamina enclosed tubes. Ultimately regenerating fibers are formed de novo, and most, if not all, of the fibers receive motor innervation. Despite extensive literature describing the morphology and physiology of the transplant, little is known about the transplanted muscle's innervation. In order to determine the size and location of the motoneuron pool of orthotopically transplanted extensor digitorum longus muscles (EDL), free muscle grafts (Carlson and Gutmann, Experientia, 30:1292, 1974) were performed on the EDL muscle of nineteen 59-day-old 129 ReJ female mice. One hundred days post-transplantation, the grafted muscles were exposed and horseradish peroxidase (HRP) paste was placed within the muscle using an endodontic file. The unoperated EDL of fifteen 156-day-old mice were also exposed and treated with HRP in order to determine the size and location of the motoneuron pool of the normal EDL. After 18-24 hours the anesthetized mice were intraventricularly perfused with normal saline, followed by cold 1% paraformaldehyde -1% gluteraldehyde in 0.1M phosphate buffer (pH 7.2). Spinal cord segments L_2-L_4 as well as the associated dorsal root ganglion were stored overnight in 30% sucrose (4°C). Serial 40 µm thick sections were cut on a cryostat and reacted for HRP reaction product using the TMB method of Mesulam (J. Histochem. Cytochem., 26:106, 1978). The motoneuron pool of the normal EDL is located in the anteriolateral motor column (Lamina IX) at the level of spinal segment L_3 and extends an average distance of 800 µm ±106. The normal muscle is innervated by 14±3 motoneurons (N=15). Although the location of the motoneuron pool of the transplanted muscle is in the anteriolateral motor column at the level of spinal segment L_3, the number of motoneurons innervating the graft is decreased to 8±4 (N=19). One of the grafts was innervated by a single motoneuron and two of the grafts had the mean number of motor units found in control muscles. Evaluation of the dorsal root ganglion at the level L_3 indicated that the grafted muscle did receive sensory innervation. While the number of motoneurons innervating the graft is reduced, it has not been determined whether all the regenerating axons have grown out of the severed nerve or whether there is a contribution due to the sprouting of nerves from surrounding intact muscles.

(Supported by NIH-NS13688 and NSF-PCM8202612.)

355.12 β-ADRENERGIC RESPONSE AND ACETYLCHOLINESTERASE IN MURINE DYSTROPHIC MUSCLE AS RELATED TO A MATURATIONAL DEFECT. K. A. Skau. University of Cincinnati; Cincinnati, OH 45267.

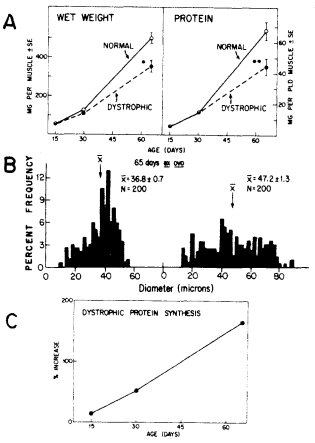
Murine muscular dystrophy has been suggested to be a maturational defect that involves a muscle membrane abnormality. To test this hypothesis, this study examined the involvement of two membrane proteins in the development of the disease in dystrophic (dy/dy) and clinically normal (+/?) mice of the ReJ/129 strain. Juvenile mice for this study were 4 weeks or younger animals bred in this laboratory as offspring of heterozygous (dy/+) parents.

Biochemical studies on the globular tetrameric (G4) form of acetylcholinesterase (AChE) were performed on extracts of hemidiaphragm (HD) and extensor digitorum longus (EDL) solubilized with a high-salt buffer containing Triton X-100. Samples of the low-speed supernatant of this extract were centrifuged on 5-20% linear sucrose density gradients and divided into 25 fractions which were assayed radioenzymatically for AChE. The distribution of AChE in juvenile mouse HD had a pattern similar to that of dystrophic muscle i.e. an enriched G1 and virtually no G4 peak. This pattern was observed in all juvenile HD of mice 4-21 days old. By contrast, newborn mice exhibited three approximately equivalent peaks of AChE which may be related to a high nerve-to-muscle ratio prior to synapse elimination. By 28 days of age all non-dystrophic mice exhibited an AChE profile similar to that of normal adults. Juvenile EDL also lacked the G4 form of the enzyme up to 3-4 weeks of age. These results support the hypothesis of a maturational defect.

The β-adrenergic agonist isoproterenol (ISO) enhances the contractility of isolated fast-twitch mammalian muscle by activating a membrane-bound β_2-adrenoceptor. In clinically normal adult mouse HD ISO (0.001-10µM) enhanced muscle contractility up to 35%. Dystrophic adult muscle was enhanced a maximum of 25%. HD from 2-week old juvenile mice of heterozygous parents were most sensitive to ISO exhibiting approximately 100% increase in force of contraction. While these results do not support the hypothesis of a maturational defect, it is possible that other factors affect the dystrophic muscle response to ISO. It is not possible to determine the relative β-adrenoceptor density in clinically normal, dystrophic and juvenile muscle from these muscle contraction studies.

355.13 ABNORMAL PROTEIN TURNOVER IN PLD MUSCLE EXPLANTS FROM GENETICALLY DYSTROPHIC CHICKENS TO ASSESS INTRAPERITONEAL DRUG THERAPY, M.S.Hudecki, C.M.Pollina* and R.R.Heffner, Depts. of Biological Sciences and Pathology, State University of New York, Buffalo, NY 14260

In spite of the controversy regarding the dystrophic genotype and possible muscle protein turnover abnormalities, the progressive and debilitating loss of functional muscle mass in affected animals(as well as humans) is a needed focus of drug intervention. Hence, our on-going study of daily intraperitoneal(IP) drug effects on Line 413 dystrophic chickens has recently included *in vitro* assays of protein turnover(i.e., synthesis) of isolated posterior latissimus dorsi(PLD) muscle explants. This fast-twitch muscle progressively loses mass and protein with age(Fig. A,*$p<0.001$,**$p<0.01$); and exhibits various histopathological lesions, including necrotic and vacuolated fibers, and increases in small and large fibers similar to that found in the pectoralis muscle(Fig.B). These abnormalities in PLD mass and histology are coincident with overt functional disability and dramatic rise in plasma creatine kinase activies. The protocol for measuring PLD synthesis(Wolitzky, B.A., et.al., under review) involved ^{3}H-tyrosine labeling *in vitro* of total and specific proteins of isolated PLD muscles from dystrophic and genetically-related Line 412 normal birds at various ages *ex ovo*. As a result, abnormal elevations in the rates of total(Fig.C) and specific protein synthesis were found in the dystrophic PLD with a 165% increment evident at 65 days. The aim of the present study is to administer selected drugs IP for 1 to 3 months and subsequently assess *in vivo* treatment to "normalize" PLD protein turnover *in vitro*. This approach is anticipated to provide objective and sensitive data relevant to dystrophic chemotherapy.



Supported by the MDA Task Force on Drug Development and NIH(NS 16219). Work of B.A. Wolitzky and J.A. Stamos gratefully acknowledged.

355.14 ALTERATIONS IN GLYCOSPHINGOLIPID BIOSYNTHESIS DURING AVIAN MYOGENESIS *IN VITRO*. E. L. Hogan, J.-L. Chien*, and K. C. Leskawa*. (SPON. by N. Banik) Department of Neurology, Medical University of South Carolina, Charleston SC 29425

Primary muscle cell cultures from the pectoral muscle of 12 day chick embryos were used to study the biosynthesis of membrane glycosphingolipids. With this system, the myogenic process can be conveniently separated into several distinct phases: an inital period during which time myoblasts are actively replicating (R phase); a stationary period during a cell fusion block, mediated by the chelation of media calcium ion (St phase); the initial burst of myoblast fusion, initiated by restoration of normal media calcium ion levels (Fi phase); the completion of myotubule formation (Fc phase); and the isolation of functional myotubes by the elimination of replicating cells by the addition of cytosine arabinoside (Fc + ARA-C phase). Either tritiated galactose or glucosamine were added in complete media to muscle cell cultures during each of these phases (20 uCi/10 cm-diameter petri dish). Following extraction and isolation by standard procedures, total glycosphingolipids were separated by thin-layer chromatography and visualized by autoradiography. During the R phase, the major unsialylated glycosphingolipid biosynthesized by myoblasts were lactosylceramide, a tetraosylceramide and a much more complex glycolipid, possibly a hexosylceramide. During the fusion block (St phase) and the initial stages of myoblasts fusion (Fi phase), a triosylceramide emerges as the major biosynthetic product, with corresponding decreases in the more complex glycosphingolipids. Also biosynthesized during these myogenic stages is a pentaosyl ceramide, tentatively identified as the Forssman hapten glycolipid. After the completion of myotube formation, triosylceramide and pentaosylceramide (the Forssman hapten) biosynthesis drastically decrease and lactosylceramide and tetraosylceramide again emerge as the major biosynthetic products. Similar changes in sialic acid-containing glycolipids (gangliosides) will also be presented.

Supported by the Medical University of South Carolina (State Appropriations for Research, 1982, 1983) and a grant from the Muscular Dystrophy Association.

355.15 EFFECT OF GLUCOCORTICOIDS ON EXCITATION-CONTRACTION COUPLING IN RAT FAST- AND SLOW-TWITCH MUSCLE FIBERS. B. Laszewski* and R.L. Ruff (SPON: S. Bledsoe). Depts. of Physiology and Biophysics and Medicine (Neurology), Univ. of Wash., Seattle, WA 98195.

Pharmacological doses of glucocorticoids induce skeletal muscle weakness and atrophy with a preferential effect on fast-twitch muscle. The possibility that decreased myofibrillar Ca sensitivity caused the weakness was investigated using the skinned fiber technique and by studying strength-duration curves. Following intramuscular treatment with either 1.5 mg/kg/day dexamethasone or saline for 14 days, rat extensor digitorum longus (EDL) (fast-twitch) and soleus (slow-twitch) muscles were excised and chemically skinned or studied *in vitro* at 37°C with a two electrode voltage clamp. Single skinned muscle fibers were isolated and tension was recorded over a pCa range of 7.0 to 4.0. The EDL pCa/tension relationship was not altered by glucocorticoid treatment, however, the soleus fibers from dexamethasone-treated animals had slightly decreased Ca sensitivity. Strength-duration studies of mechanical threshold showed a similar effect. The threshold depolarizations to induce contraction for soleus fibers from dexamethasone treated rats were increased compared to control, while the strength-duration relationship for EDL fibers was not altered by glucocorticoid treatment. Both results suggest that glucocorticoid treatment reduced the calcium sensitivity of soleus but not EDL fibers. Since the twitch and tetanic force produced by soleus muscles was not reduced by glucocorticoid treatment, the reduced Ca sensitivity in soleus fibers is probably not physiologically important. Therefore, decreased Ca sensitivity does not appear to be the mechanism of the selective fast-twitch fiber weakness induced by glucocorticoid treatment. Supported by NIH grants NS00498 and NS16696.

355.16 PHOSPHOLIPID METHYLATION IN SKELETAL MUSCLE. R. W. Kuncl, Y. Kishimoto* and D. B. Drachman. Department of Neurology, Johns Hopkins Sch. of Med. Baltimore, MD 21205

Phospholipid methylation is an enzymatic reaction that occurs in a wide variety of tissues and may have important implications for the biology of cell membranes. It is thought to modulate such vital cellular processes as calcium transport, receptor insertion and function, and membrane microviscosity. Since these processes are fundamental to the function of muscle cells, and are thought to be altered in disease states, we have studied the properties of phospholipid methylation in skeletal muscle.

Rat leg muscles were homogenized, and preparations of whole muscle, cell sap, and sarcoplasmic reticulum (SR) were made. Methyltransferase activity was assayed by measuring the incorporation of labeled methyl groups from S-adenosyl-L-[methyl-^{3}H]-methionine into phospholipids. The mono-, di-, and tri-methylated derivatives of phosphatidylethanolamine were separated by thin layer chromatography (TLC) and measured by scintillation counting.

We characterized several features of phospholipid methylation reactions in adult rat leg muscle:

1) As in other tissues, two different phospholipid methyltransferases (PMT-I and PMT-II) were present, having different substrate affinities and reaction products. The high affinity enzyme, PMT-I, catalyzes the addition of the first methyl group to phosphatidylethanolamine, and PMT-II adds subsequent methyl groups.

2) PMT-I activity was membrane-bound in muscle, being enriched 23-fold in the SR fraction compared to whole muscle homogenate; activity was absent in cell sap.

3) The K_m for PMT-I was 2.2 μM, and the K_m for PMT-II was 17 μM. Both reactions were optimal at pH 8.0-8.5.

4) The PMT-I reaction was blocked by several methyltransferase inhibitors, including S-adenosylhomocysteine (K_i = 0.83 μM).

5) The specific activity of PMT-I in SR was 3.0 ± 0.3 pmoles/mg protein/hr. This is higher than activity reported for rat RBCs and brain, but less than that reported for liver and adrenal.

These observations demonstrate the presence of phospholipid methylation activity in skeletal muscle. The data will be important for further studies of the role of phospholipid methylation in skeletal muscle function and its possible alteration in skeletal muscle disorders.

356.1 TOPOGRAPHICAL PROJECTION OF THE CEREBRAL CORTEX TO THE SUBTHALAMIC NUCLEUS. S. Afsharpour* and S. T. Kitai (SPON: D. Tanaka). Dept. of Anatomy, Michigan State University, E. Lansing, MI 48824.

Rat corticosubthalamic projections were investigated using the tritiated amino acid autoradiographic axonal tracing technique. A mixture (1:1) of two amino acids (i.e. Proline and Leucine or Proline and Lysine) were stereotaxically injected (0.25-0.4 µl) unilaterally into the portions of the frontal, parietal and occipital cerebral cortices. Post injection survival times ranged from 4 to 7 days. Following cardiac perfusion with 4% formaldehyde in phosphate-buffered saline (pH 7.2), brains were removed and post-fixed in 4% formaldehyde with 30% sucrose for 12 to 24 hrs. Frozen sections of 25-30 µm in thickness in the frontal, sagittal and horizontal planes were prepared, mounted on subbed slides, dehydrated through graded alcohols, deffated through fresh xylene, rehydrated and then air dried. The slides were dipped in Kodak NTB-2 nuclear emulsion and exposed for 4 to 12 weeks at 4°C. Slides were developed with D-19 (Kodak) at 18-20°C, washed, fixed, counterstained with cresyl violet and examined with both light- and dark-field microscopy.

Silver grains could only be traced into the ipsilateral subthalamic nucleus (STH). In general, projections from the lateral agranular cortex were much heavier than those from the medial agranular cortex. The rostral part of the medial agranular frontal cortex projects throughout the ventral two-thirds of the medial half of STH. The lateral agranular frontal cortex projects to the lateral portion of the rostral two-thirds of STH. The area between the medial and the lateral agranular cortex projects mainly to the medial half of STH. More sparse projection from this area of cortex was also found in a medial portion of the lateral half of caudal STH. The caudal part of the medial agranular frontal cortex projects to the dorsolateral part of the caudal two-thirds of STH, and a sparse projection was also observed in the ventral part of the medial half of caudal STH caudally. The caudal part of the lateral agranular cortex projects to the ventral aspect of the middle third of STH. Injections into granular areas of the cortex (e.g. somatosensory, visual and association areas) did not result in labeling in STH.

These results suggest that only the frontal agranular cortex projects to STH in the rat. The cortico-STH projection is ipsilateral and terminates in a topographical manner in all parts of STH.

(Supported by NIH Grant Ns 14866 to S.T.K.).

356.2 A NEWLY DISCOVERED PROJECTION FROM THE SUBTHALAMIC NUCLEUS TO THE CAUDATE NUCLEUS AND PUTAMEN IN THE CAT. R.M. Beckstead, Dept. of Anat., Univ. of Virginia Schl. of Med., Charlottesville, VA 22908

For several years, the domain of subthalamic nucleus connections was considered to involve simply an input from the external segment of the globus pallidus and an output to both pallidal segments. More recently, STN projections to the pars reticulata of the substantia nigra have been described (Carpenter et al., Brain Res., 224:1, 1981). Because STN is thus strategically located to control pallidal and nigral outflow and because of clinicopathological reports in cases of hemiballismus, the presently dominant concept of STN function is that it acts to "suppress" the output of the corpus striatum. The recent observation of a projection from STN to the pedunculopontine nucleus (Jackson & Crossman, Neurosci. Letters, 22:17, 1981) is compatible with the notion that STN has its major influence on corpus striatal output since the pedunculopontine nucleus itself is a target of such output.

The present data show that STN projects also to the "input side" of the corpus striatum, that is, the caudate nucleus (CD) and putamen (PU). Small (10 to 15 nl) injections of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP, 1.5% in saline) were made bilaterally in various zones of CD and PU in 8 cats. After two days survival, the cats were killed by transcardial perfusion and the brains processed with a modification of Mesulam's tetramethylbenzidine reaction to reveal antero- and retrogradely transported WGA-HRP. Regardless of the location of the WGA-HRP deposit, a variable number of retrogradely labeled neurons are present in STN, scattered throughout its rostrocaudal extent. However, a crude mediolateral topography appears to exist such that medial CD deposits of WGA-HRP label cells concentrated toward the medial portion of STN and lateral CD deposits label cells more laterally located. The labeling in most STN cells is modest compared with the usually vivid labeling of cells in the pars compacta of the substantia nigra and thalamus suggesting that the striatal projection of STN cells may represent a collateral branch of the major axon ending in the globus pallidus. The appearance of diffuse, extrasomal, HRP-reaction product in some parts of STN gives the impression of a terminal distribution from striatal efferent axons descending in the cerebral peduncle.

Thus, the present results demonstrate that the STN is perhaps reciprocally connected with both the striatum as well as the pallidum. Therefore, conceptions of the functional role of STN must now be modified to include its probable influence on the "input side" as well as the "output side" of the corpus striatal circuitry.

Supported by NIH grant NS17827.

356.3 POSTNATAL ONTOGENY OF AFFERENT INPUT TO THE VENTRAL ANTERIOR AND VENTRAL LATERAL THALAMIC NUCLEI IN CATS. R.A. Gazzara, M.S. Levine and C.D. Hull. Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

The postnatal ontogeny of afferent input to the ventral anterior-ventral lateral (VA-VL) thalamic nuclei from the motor cortex (Cx), entopeduncular nucleus (ENTO), and deep cerebellar nuclei (Cbl) was assessed by anatomical and physiological methods.

Lectin-bound horseradish peroxidase (WG-HRP) was injected into the VA-VL thalamic nuclei of neonatal kittens (<72 hr postnatal) and adult cats (>1 yr). In both neonatal and adult cats, retrogradely labeled cells were found in the pericruciate Cx, ENTO, and Cbl. Labeled cells in the Cx were medium-sized pyramidal cells located mainly in layers 5 and 6. Labeled ENTO cells were medium-sized fusiform cells while labeled Cbl cells were medium-to-large fusiform neurons located in the dentate, interpositus, and fastigial nuclei. Preliminary morphological analysis suggests that these neurons undergo postnatal growth as well as displacement due to volumetric expansion of the brain.

We also have obtained intracellular recordings from VA-VL neurons in kittens ranging in age from 3 to 37 days. Analysis of the records suggests that the responses evoked by stimulation of Cx, ENTO, and Cbl (brachium conjunctivum) do not differ across age groups and are similar, in terms of initially evoked inhibition or excitation, to those we obtained in adult cats. With the data pooled across age groups, Cx stimulation evoked primarily an initial inhibition (95%; 38/40 responsive cells). The majority of cells responded to ENTO stimulation with an initial inhibition (66%; 21/32 cells) and to Cbl stimulation with an initial excitation (73%; 16/22 cells). In contrast, response latencies show a progressive decrease from early neonatal age to adulthood. The mean latency to the initial inhibition evoked by Cx stimulation for each age group was as follows: 28.2 msec (3-10 days; n=11 cells), 19.0 msec (11-20 days; n=12), 13.3 msec (21-37 days; n=15) 7.2 msec (adult, n=72).

These data suggest that projection axons to the VA-VL nuclei from Cx, ENTO, and Cbl are formed prenatally and that the inputs from these areas are functional at an early neonatal age. The major maturational effects appear to be neuronal growth as well as an increase in conduction velocity, presumably a result of axonal myelination and/or an increase in axonal diameter. These results also extend the finding that connectivity within the basal ganglia is functional at an early neonatal age, to include a major efferent system of the basal ganglia.

Supported by USPHS grants HD 07032 and HD 05958.

356.4 THE ORGANIZATION OF INTRALAMINAR THALAMOSTRIATAL PROJECTIONS IN THE CAT STUDIED WITH WHEAT GERM-HRP AND AUTORADIOGRAPHY. M. Marshall* & R.M. Beckstead (Spon: J.R. Morse). Dept. of Anat., Univ. of Virginia Schl. of Med., Charlottesville, VA 22908.

The organization of intralaminar thalamostriatal projections was analyzed in the cat by retrograde cell-labeling with wheatgerm agglutinin conjugated to horseradish peroxidase (WGA-HRP, 1.5% in saline), and by anterograde, autoradiographic, axon-labeling with ^{3}H-amino acids. To identify the intralaminar neurons that project to the striatum, small deposits of WGA-HRP were placed in various regions of the caudate nucleus (CD), putamen (PU) and nucleus accumbens (AC) and the brains were processed according to a modification of the tetramethylbenzidine method. To examine the intrastriatal axonal distribution, ^{3}H-proline-leucine (1 to 1 mixture in saline; 50µCi/µl) was injected into each of the intralaminar nuclei and the brains were processed for autoradiography.

All intrastriatal terminal distributions from the intralaminar nuclei display irregular discontinuities to varying degrees. The intralaminar nuclei as a group project topographically to the striatum. The dorsalmost of the group, the central lateral nucleus (CL) projects most densely to the dorsal half of the head and body of CD and very sparsely to the dorsal half of PU. The ventralmost, central medial nucleus (CeM) projects mainly to the ventral (fundus) portion of the CD and PU rostrally, including sparsely the lateral part of AC, and to the ventral part of the body of CD. The paracentral nucleus (PC), located between CL and CeM, projects mainly between these two intrastriatal distributions, to the middle dorsoventral region of the head and body of CD and the ventral part of PU rostrally. The large, centromedian-parafascicular complex (CM-PF) projects densely to all parts of CD and PU and sparsely to AC. The CM-PF projection itself is organized topographically in the dorsoventral and mediolateral dimensions. Axons from all the intralaminar nuclei travel dorsolaterally to enter the internal capsule (IC). However, some CM-PF axons instead descend with the retroflex bundle, turn laterally in the ventral tegmentum and course over the subthalamic nucleus (STN) before gaining IC. Some such axons appear to terminate STN.

Retrograde labeling of intralaminar cells confirms the general topographic principles revealed autoradiographically but reveals also the following, second-order principal of organization. Small deposits of WGA-HRP in various parts of the striatum often label intralaminar cells arranged in clusters that can sometimes be widely separated from one another even within the same nucleus. Such clustering has been noted previously for projection neurons in the principal thalamic nuclei. The significance of this clustering, like that of the terminal discontinuities, remains obscure.

Supported by NIH grant NS 17827.

356.5 THALAMIC NUCLEI OTHER THAN INTRALAMINAR THAT PROJECT TO THE STRIATUM DEMONSTRATED BY TRANSPORT TECHNIQUES IN THE CAT. K. Kersey* & R.M. Beckstead (Spon: R.J. Krieg) Dept. of Anat., Univ. of Virginia Med. Schl., Charlottesville, VA 22908.

In the course of investigating intralaminar thalamostriatal projections in the cat, we found that some thalamic nuclei outside the internal medullary lamina project to the caudate nucleus (CD), putamen (PU) and nucleus accumbens (AC). By making a number of small (5-15nl) deposits of horseradish peroxidase conjugated to wheatgerm agglutinin (WGA-HRP, 1.5% in saline), well-localized in several regions of the striatum, we were able to label a small number of cells in the ventral anterior (VA), ventromedial (VM) and lateral posterior (LP) nuclei and a relatively large number of cells in the posterior paraventricular nucleus (PV), the rhomboid nucleus (RH) including its winglike extension, the interanterodorsal nucleus, and in cases of AC injection, the paratenial nucleus (PT). Another group of labeled cells occurs in a region lateral and dorsal to the stria medullaris; such cells are not readily attributable to the mediodorsal nucleus or LP, but instead appear to constitute a confluence of labeled cells of PV and the central lateral nucleus.

Analysis of the cell-labeling with respect to the specific locations of the intrastriatal enzyme deposits reveals the following. The labeled VA cells, which are consistently located anteriorly in a narrow alignment along the oblique medial border of the nucleus, occur after all injections in the head of CD. After rostral PU injections, no VA cells are labeled, but a number of cells in the anterior VM are HRP-positive. Cell-labeling in LP is inconsistent and the very few cells that do occur are randomly and widely scattered in its medial subdivision. Relative to their small size, RH and PV are most abundantly labeled after medially placed deposits in the head of CD, PU and AC. After AC injections, labeled cells also occur in PT. It is noteworthy that WGA-HRP deposits in the caudal part of PU fail to label any thalamic neurons except a few in the centromedian-parafascicular complex.

Perhaps the most interesting cell-labeling after medially placed CD and AC deposits is that lateral and dorsal to the stria medullaris. Such cells are morphologically similar to and continuous ventrolaterally and ventromedially with labeled cells in, respectively, the central lateral nucleus and PV. Autoradiography after ^{3}H-amino acid injection of this cell group confirms its axonal distribution in medial CD and accumbens, and shows further that like the intralaminar thalamostriatal projections, the distribution is non-homogeneous.

These data indicate that RH, PV, PT and a region lateral to the stria medullaris are at least as intimately associated with the striatum as the intralaminar group of nuclei.

Supported by NIH grant NS 17827.

356.6 DIRECT PROJECTIONS OF THE GLOBUS PALLIDUS TO THE MEDIAL THALAMUS IN THE RAT. T. Hattori and T. Sugimoto. Dept. Anat., Fac. Med., Univ. Toronto, Med. Sci. Bldg., Toronto, Ont. M5S 1A8 Canada.

Entopeduncular nucleus (Ep) is regarded as the exclusive pallidal source of innervation to the thalamic core nuclei (VAL, CM-Pf and lateral habenula). In contrast, thalamic terminations of the external pallidum i.e. globus pallidus (GP) neurons are reported only in the shell of the thalamus — thalamic reticular nucleus (Nauta, '79). We report a novel GP projection to the thalamus in the rat. Injections of HRP into the medial thalamus produced retrograde cell labeling as well as anterograde fiber labeling in the GP and Ep. HRP-labeled neurons in the GP were diffusely scattered throughout the GP, consisted of neurons with fusiform or triangular shape and had an average cell diameter of 10-20 μm. On the other hand, HRP-labeled Ep neurons were usually multipolar and 20-28 μm in diameter, which corresponded to Ep neurons heavily labeled following HRP injections in the lateral habenula. 3H-leucine injections in the GP revealed sparse anterograde radiolabeling in the medial thalamus as well as extensive radiolabeling in the subthalamic nucleus and substantia nigra. In the medial thalamus, accumulation of silver grains was observed in the paraventricular nucleus bilaterally with an ipsilateral predominance and much less significantly in the adjacent part of parafascicular nucleus. These thalamic sites were examined electron microscopically; following HRP injections in the GP, several profiles of HRP-labeled terminal boutons were seen in the medial thalamus. Since these medial thalamic neurons are known to project massively to the caudoputamen and nucleus accumbens, the present GP-thalamic projections may contribute to the output part of the corpus striatum in its thalamic loop system. (Supported by the Medical Research Council of Canada.)

356.7 SUBDIVISIONS OF MOTOR RELATED THALAMIC NUCLEI IN THE CAT BASED ON THEIR CONNECTIVITY AND ULTRASTRUCTURE. K. Kultas-Ilinsky and I.A. Ilinsky. University of Iowa College of Medicine, Iowa City, IA, 52242

Subdivisions of motor related thalamic nuclei (VA, VL, VM) in the cat are presently based on cytological differences as well as on the topography of their connections with the frontal cortex. However, neither of these criteria outline precisely the boundaries between the VA, VL and VM. In the present study we made an attempt to outline these subdivisions of ventral thalamus on the basis of topography of cerebellar and basal ganglia projections.

Thalamic afferents from the dentate nucleus of cerebellum and entopeduncular nucleus were studied by means of light microscopic autoradiography on serial sagittal sections after injections of tritiated leucine into these nuclei. The location and spatial relationships of the two projection systems in the thalamus were compared and mapped at the same sagittal planes.

The results demonstrate that the main pallidal and dentate projection zones in the thalamus are entirely segregated along the anterior-posterior axis. The main territory of pallidal projections is confined within the larger ventral medial part of the VA while the dentate projection field is located exactly posteriorly to it within the confines of the VL. Although a portion of dentato-thalamic projections does go to the VA the territory occupied by them is distinct from that controlled by pallidal projections as it is located in the dorsal-lateral part of the VA nucleus. Based on these findings we suggest to consider as the VA only that part of the nucleus which receives the pallidal projections. Consequently, the part of the VA receiving the dentate projections can be considered as a part of the VL instead. Such delineation is supported also by the available data on the thalamo-cortical projections of the two VA regions.

The VM, on the other hand, can be best delineated on the basis of distribution of nigrothalamic afferents as described earlier (Ilinsky et al., Appl. Neurophysiol. 45:230-237, 1982). Unlike the VA and VL, the VM is the only nucleus in the ventral group which receives the projections from both basal ganglia (substantia nigra and entopeduncular nucleus) and cerebellum (dentate and fastigial nuclei).

Qualitative and quantitative analysis of synaptic organization of the VA, VL and VM indicates that there are definite differences in regard to the types and relative proportions of synaptic boutons in the three nuclei which can be directly related to the differences in afferent inputs as seen at light microscopic level.

This study was supported by a grant from Am. Parkinson Disease Association and NIH grant RO1NS1980.

356.PO MICROINJECTIONS OF RETROGRADE FLUORESCENT TRACERS IN THE VENTRAL PALLIDUM OF RAT LABEL NEURONS AT THE MEDIAL EDGE OF THE SUBTHALAMIC NUCLEUS. L. Heimer[1,2], G.F. Alheid[3], and L. Zaborszky[1]. 1. Departments of Neurology, 2. Neurosurgery, and 3. Department of Behavioral Medicine and Psychiatry, Clinical Neurosciences Research Center, Univ. of Virginia School of Medicine, Charlottesville, Va. 22908.

Fluorescent tracer filled micropipettes were implanted in the globus pallidus and ventral pallidum of male albino rats. This allows small (ca. 500 μm) tracer injections without labeling of overlying structures (Alheid and Carlsen, 1982).

Injection placements of granular blue (GB) or fast blue within the ventral pallidum were confirmed by immunostaining brain sections adjacent to those used for analysis of retrograde label. Only when the injection sites were within the enkephalin or substance-P rich neuropil that is characteristic of both dorsal and ventral pallidal areas, were cells retrogradely labeled at the level of the subthalamic nucleus.

After injections in the subcommissural part of the pallidum, labeled neurons were found at the medial edge of the cerebral peduncle and in continuity with the most medial and ventral tip of the subthalamic nucleus. Pallidal injections just dorsal to the anterior commissure in the anterior tip of the globus pallidus label neurons in the medial edge of the subthalamic nucleus in a distribution that overlaps the most dorsal cells labeled after ventral pallidal injections.

These results indicate that the topographical subthalamic-pallidal projection system includes projections to ventral pallidum and that the ventral projecting subthalamic neurons invade the posterior lateral hypothalamus.

This work was supported by NIH grant R NS1774303.

357.1 STRIATAL NEURON RESPONSE TO LOCALLY PERFUSED ETHANOL. E.P. Schoener. Department of Pharmacology, Wayne State University, School of Medicine, Detroit, MI 48201.

Although cellular effects of ethanol (ETOH) have been studied with considerable interest in recent years, the variety of manifestations observed under different conditions has not provided a systematic understanding of its mechanism(s) of action. In the present study, push-pull perfusion (PPP) and extracellular single unit recording techniques were combined to investigate the effects of discretely applied ETOH on the function of striatal neurons.

Adult, male Sprague-Dawley rats anesthetized with urethane (1 gm/kg) were employed in these experiments. They were cannulated, intubated and mounted in a stereotaxic device according to standard procedure. A limited craniotomy permitted access for steoreotaxic placement of the PPP cannula (0.7 mm O.D.)and the recording microelectrode into the striatum with separate manipulators. Artificial CSF was continuously perfused through the push-pull cannula at a rate of 25 µl/min. While observing the activity of single striatal units, ETOH was added to this perfusion fluid at various concentrations (10^{-9} to 10^{-4}M), for 3 min test periods, after 10 min control recording periods. At the two highest concentrations studied (10^{-5} and 10^{-4}M), neurons were almost invariably depressed, while the converse was true at the lowest concentrations used (10^{-8} to 10^{-9}M). The depression seemed to peak most specifically at 10^{-6}M (-58%); the facilitation did so at 10^{-8}M (+63%). In the midrange, along with both excitatory and inhibitory responses, there were a number of apparently insensitive units and bimodally reactive neurons (excitation followed by depression).

The striatal neurons examined in these studies with PPP were responsive to locally perfused ETOH over an extremely wide dose range. The consistent neuronal activation achieved with very low concentrations of ETOH suggests the possibility that physiologic levels of ETOH may normally play a role in the regulation of CNS excitability.

357.2 BASIS OF ETHANOL EFFECTS ON FIRING PATTERNS OF APLYSIA NEURONS DETERMINED BY VOLTAGE CLAMP. M.H. Schwartz* (SPON: R.L. Katz). Neuroscience Program, Brain Research Inst., UCLA, Los Angeles, CA 90024.

We investigated ethanol (EtOH) effects on identified neurons of the marine mollusc Aplysia californica. EtOH caused profound changes in cell firing patterns. In R2, a silent cell, 4% EtOH initiated bursting. In R15, a bursting pacemaker, 4% EtOH eliminated bursting and the cell began beating with a high firing frequency. It has previously been shown that the rhythm underlying bursting is present in the absence of cell spiking (Junge,D. and Stephens, C., J.Physiol.(Lond.),235:155,1973). Changes in firing pattern induced by EtOH were therefore likely due to a change in subthreshold phenomena. Conventional 2-electrode voltage clamp methods were used to study the basis of EtOH effects on the subthreshold or pacemaker currents.

The steady-state current-voltage (I-V) relation of bursting pacemakers was determined by measuring the currents elicited 5 sec. after clamping the membrane potential to values between -80 and -25 mV from a holding potential of -55 mV. In 4% EtOH the I-V showed an apparent decrease in n-shape or slow inward current (I_{si}) and an apparent increase in outward current (I_{out}). Ion substitution and drug blocking experiments were performed to show the basis of the change in the I-V. The EtOH effect was not dependent on a decrease in the sodium (Na) component of I_{si} since 4% EtOH abolished the residual I_{si} and increased I_{out} in a nearly Na-free (10mM) solution. The effect of EtOH on I_{out} alone was studied by eliminating I_{si} in a 10mM Na, low calcium (Ca) solution in which residual Ca-current was blocked with one of the divalents--nickel, cadmium or manganese. I_{out} was substantially increased under these conditions by 4% EtOH. Blockage of the inward currents as above and also the fast outward current, I_A, by 4-aminopyridine (4-AP) and the delayed rectifier current, I_K, by tetraethylammonium (TEA) allowed specific study of the leakage current. 4% EtOH decreased the leakage current. The EtOH induced increase in I_{out} was therefore a direct effect on the voltage-activated potassium currents. The possibility that all of the EtOH effects on net membrane current could be attributed to the increase in I_{out} (which masks I_{si}) was then tested. Outward potassium current was eliminated by nystatin loading of the cell with cesium following the procedure by D. Tillotson (PNAS,76:1497,1979). The test solution further contained 10mM Na, 100mM TEA and 4mM 4-AP so that the measured current was the Ca-component of I_{si}. This I_{si} was substantially decreased in 4% EtOH. The above methods can be used to determine the characteristic amounts of individual pacemaker currents in identified neurons. This knowledge combined with our results can explain changes of endogenous firing patterns in alcohol containing solutions.

357.3 INFLUENCE OF ACUTE AND CHRONIC ETHANOL ADMINISTRATION ON THE INHIBITORY ACTIONS OF ETHANOL IN RAT VAS DEFERENS. K. H. DeTurck* and L. A. Pohorecky* (SPON: R. C. De Groof) Center of Alcohol Studies, Rutgers University, New Brunswick, NJ 08903

Ethanol produces a dose-dependent inhibition of norepinephrine(NE)-induced contractions in the rat vas deferens. The possibility was investigated that acute or chronic ethanol treatment of rats might modify this effect of ethanol in vitro. In each experiment, cumulative dose-response curves of isotonic contractions induced by NE were recorded before and after the 15 min exposure of the preparations to 181.3mM ethanol in Krebs buffer. Tissues obtained from untreated Sprague-Dawley rats demonstrated 40-50% reductions in their contractile response to exogenous NE following incubation with this concentration of ethanol.

In comparison to the effects of saline administration, acute treatment of rats with 2.5 g/kg ethanol 30 min before sacrifice reduced (42%) the inhibitory effect of ethanol on the vas deferens response to the lowest concentration of NE (10^{-10}M). Contractions elicited by subsequent NE additions, however, were similar in both treatment groups. Chronic administration of ethanol, 8-13 g/kg per day for 14 days, resulted in significant tolerance to the inhibitory action of this drug on muscular contractions evoked by NE in vitro. Tissues of ethanol-dependent rats exhibited decreases of roughly one-half those of the isocaloric, maltose dextrin, and water control groups in response to 181.3mM ethanol. The difference, between groups, in contractile responses with ethanol exposure was greatest at moderate NE concentrations (10^{-9} to 10^{-7} M). These results indicate that ethanol exposure in vivo can produce some degree of tolerance in vitro.

Supported by USPHS grants 00045 and 04238.

357.4 A TECHNIQUE FOR CORRELATING ETHANOL'S EFFECTS ON MOTOR PERFORMANCE AND ON SINGLE NEURON ACTIVITY IN ETHANOL-PREFERRING (P) AND NON-PREFERRING (NP) RAT STRAINS. S. M. Sorensen, J. K. Chapin, L. Lumeng, T. K. Li, and D. J. Woodward. Dept. of Cell Biology, Univ. of Texas Hlth. Sci. Ctr. at Dallas, Texas 75235, and Indiana Univ., Bloomington.

The P and NP rat strains were originally bred for differences in ethanol preference (Lumeng et al. in: Currents in Alcoholism. Vol. 3, New York: Grune & Stratton, 1978). Marked differences have also emerged in their sensitivity to the effects of acute ethanol on motor performance in that P rats are less impaired than NP rats. We have recently begun to investigate the electrophysiological correlates of the differential sensitivity to acute ethanol in these rat strains.

Our aim has been to directly correlate the effects of ethanol on motor behaviors with its effects on specific neurophysiological systems. Previous experimental strategies have often employed anesthesia which eliminates behavior, or have evoked confounding behavioral and electrophysiological arousal signs by handling the animals while administering ethanol.

To circumvent these problems, we have developed techniques for recording from single cortical neurons in awake, unrestrained rats while simultaneously videotaping their behavior. With these techniques, neuronal responses to specific sensory stimuli can be correlated with precisely defined behaviors (Chapin et al. Exp. Neurol. 72: 1982). We have also developed a method for delivering ethanol into the peritoneum with a permanently implanted catheter which is routed subcutaneously to the recording headstage and is connected via the wiring harness to a remote syringe delivery system. This allows for ethanol administration without disrupting ongoing behavior.

In preliminary studies with the P and NP rats we have been able to record marked, dose dependent differences in the effects of ethanol on the response characteristics of somatosensory neurons to stimuli which are presented by pulses applied to indwelling electrodes in the forepaw during specific, defined behavioral states (i.e. quiet resting; treadmill locomotion). These differences can be seen at doses less than 0.3 gm/kg of ethanol. We believe that this methodology holds considerable promise for establishing some of the neuronal substrates which underlie the behavioral impairments seen in acute ethanol intoxication.

Supported by grants NIAAA AA05194-01, NIAAA 03901, and the Biological Humanics Foundation.

357.5 EFFECTS OF ETHANOL (E) ON SPONTANEOUS ACTIVITY OF DORSAL RAPHE (DR) NEURONS IN VITRO. N-S. CHU AND C. L. KEENAN. (SPON: S.van den Noort). Dept. Neurology, UCI Sch. Med, Irvine, CA 92717.

Midbrain DR neurons, especially those identified as serotonergic, are thought to be functionally important as mediators or modulators of behavior (Aghajanian, Serotonin and Behavior, 1973). Serotonin also is known to mediate the effects of many psychoactive drugs. Since ethanol is one of the oldest and most used psychoactive substances, it is important to establish its effects on serotonergic cells. To avoid systemic and most synaptic effects following systemic administration of E, the brain slice preparation is useful to study the direct action of E on serotonergic neurons. The purpose of this study was to evaluate spontaneous activity of DR cells recorded from in vitro slice preparations and to determine the effects of E on these cells. Brain stem slices (400 um thick) containing DR nucleus cells were obtained from 38 male Sprague-Dawley rats (150-200 g), maintained at 37°C, and perfused with artificial CSF at a rate of 2-4 ml/min. Chamber atmosphere and media were saturated with 95% O_2 and 5% CO_2. Single unit extracellular recordings were made with glass microelectrodes and conventional AC recording techniques. Spontaneous activity was recorded from more than 131 DR cells. Sixty-eight % of the DR cells had spontaneous firing rates of $<5/s$ ($\bar{x}$ = 2.7/s). The remaining cells had discharge rates of $>5/s$ ($\bar{x}$ = 11.7/s) Effects of E on DR neurons was dose dependent. Ethanol solutions of 50 mg/ml resulted in an increase of firing rate ($\bar{x}$ = 89%) for all cells studied. The mean firing rate decreased by 63% in 80% of the cells perfused with 100 mg/ml E. The majority of cells exposed to 200 mg/ml E displayed bursting discharges. Ethanol solutions of 100 and 200 mg/ml increased the durations and decreased the amplitudes of the action potentials of DR cells. Our results provide evidence for a direct effect of ethanol on DR neurons that is biphasic and dose-dependent. At low concentration the effects of E appear to be excitatory, while at intoxicating concentrations (100 mg%) the effects are primarily inhibitory. At even higher concentrations (200 mg%), the foremost effects of E are the appearance of bursting discharges often followed by complete cessation of activity. Our results confirm the findings of Mosko and Jacobs (Neurosci. Ltrs. 2:195, 1976) that the spontaneous activity of DR neurons can be studied in slice preparations and is similar to that observed in vivo. Furthermore, our findings are consistent with the hypothesis that the effects of ethanol are dose-dependent and biphasic at the cellular level (Kalant and Woo, Pharmacol. Ther. 14: 431, 1981).

(Supported by NIAAA grant 1-K02-AA-0049 to N-S Chu)

357.6 PROLONGED BIPHASIC EFFECTS OF ACUTE ALCOHOL INTOXICATION ON LIMBIC EXCITABILITY. Henry Lesse. Department of Psychiatry and Brain Research Institute, School of Medicine, University of California, Los Angeles, Ca. 90024

This study investigated the time course of alterations in the electrical excitability of limbic structures, important in the regulation of emotion, following acute ethanol administration. A principle aim was to characterize electrophysiolgical events in the limbic system during recovery from the sedating effects of acute alcohol intoxication. Current thresholds for electrically evoked septal, hippocampal and amygdalar afterdischarges (AD) were determined in cats prepared with an indwelling jugular cannula and multiple brain electrodes. Threshold tests were conducted in a counterbalanced order, at post-injection intervals of 5 minutes to 24 hours following 1.6 G/kg (15% intravenous ethanol, via infusion pump at 3ml/min). The effects of alternating saline and drug treatments, separated by at least 48 hours, were compared.

Results indicate that a single intravenous ethanol administration produces, over a 24 hour period, biphasic changes in the electrical excitability of major limbic structures. A significant elevation in afterdischarge threshold was detected within 5 minutes. Peak effects (73% increase in mean AD threshold) occured within 1 hour and then gradually declined over the following 8 hours. At 16 hours the opposite response to ethanol - a decrease in mean AD threshold - was found at the same limbic stimulation sites. This excitatory effect persisted during the subsequent 24 hour post-injection interval. The drug-induced afterdischarge threshold changes proved significantly greater at the amygdala and septal area than at the hippocampus during test intervals of 5 minutes to 8 hours. In addition, ethanol induced biphasic changes in AD duration at most test sites. Propagation of electrically evoked afterdischarges from limbic structures to the neocortex was blocked and convulsant behaviors were attenuated during post-injection intervals of 5 minutes to 8 hours. Deficits in performance of a bar pressing task, in general, paralleled the course of drug-induced AD threshold changes. There was an initial failure to respond, with subsequent return to normal task performance during reversal of the initial AD threshold elevations. There were individual variations in both the onset and the duration of the secondary excitatory phase.

These results suggest that time-dependent, dual effects of ethanol on the excitability of limbic structures may be important both in mediating recovery from the symptoms of acute intoxication and in determining the onset of subsequent alcohol use.

(Supported by grants NIAAA 3513 and RR 5756)

357.7 BODY TEMPERATURE MODULATES ETHANOL-INDUCED INTOXICATION IN MICE. J.R. Wenger, D.A. Finn*, G.G.A. Galleisky*, M.B. Bolger*, and R.L. Alkana. Institute for Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA 90033.

Previous studies have demonstrated that the sensitivity of the brain to ethanol-induced depression varies with temperature. These studies estimated the wake-up rectal temperatures of intoxicated mice tested at various ambient temperatures by extrapolating from simultaneously measured rectal temperatures of a separate group of intoxicated mice. The present study employed improved apparatus and procedures to replicate and extend this work by investigating the relationship between these variables in the same animals. Drug naive, male C57BL/6J mice (n=11 per temperature) weighing 15 to 22 g at testing were housed 5 per cage on a 12 hour light-dark cycle (0700 on) in a room thermostatically maintained at 21 ± 1 degrees C for 1 week before testing. Rectal temperature was recorded immediately before each animal was injected i.p. with 3.6 g/kg ethanol (20%w/v) between 1030 and 1130 hours and again when it regained its righting reflex. The mouse was then killed and its brain removed and prepared for assay of ethanol concentration. After losing their righting reflex, the mice were placed in v-shaped sleep trays within an environmental chamber maintained at 13, 15, 17, 21, 30, 34, or 35 degrees Celsius. One temperature was investigated per day. As in previous studies, ambient temperature strongly influenced rectal temperature, sleep-time, and the concentration of brain-ethanol at awakening. The rectal temperature at awakening was significantly correlated with sleeptime (r=.73) and negatively correlated with the brain-ethanol concentration (r=-.57). Over this temperature range, sleeptime monotonicly increased 103% with ambient temperature from a low of 37.6 ± 6.9 minutes at 15 degrees to a high of 86.7 ± 5.6 at 35 degrees. Wakeup rectal temperature increased from 34.1 ± .3 to 39.0 ± .2 degrees while wakeup brain ethanol concentration decreased from 3.62 ± .08 to 3.24 ± .12 mg/g. These data suggest that rectal temperature (and presumably brain temperature) determines the sensitivity of the brain to ethanol since animals which were cooler awakened sooner despite having higher brain concentrations of ethanol. The strong relationship between body temperature at awakening and sleeptime further supports the hypothesis that temperature modulates membrane mediated effects of ethanol. (Supported by grant R01 AA055234, NIAAA.)

357.8 HYPERBARIC EXPOSURE ATTENUATES DEVELOPMENT OF ETHANOL-INDUCED PHYSICAL DEPENDENCE IN MICE: PRELIMINARY STUDY. R.L. Alkana, D.A. Finn*, G.G.A. Galleisky*, J.R. Wenger and P.J. Syapin. Institute for Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA 90033.

Exposure to hyperbaric helium-oxygen reduces the acute depressant effect of ethanol and precipitates and exacerbates withdrawal in ethanol dependent animals. The present experiment studied the effect of hyperbaric treatment on the development of ethanol-induced physical dependence. Adult, drug-naive C57BL/6J male mice were housed individually and given ethanol containing liquid diet (BioServ) as the sole source of nutrients and fluid (35-40% ethanol derived calories) for 10-11 days. Experimental animals (n=7) were housed in chambers kept at 12 atmospheres absolute (ATA) helium-oxygen during dependence acquisition. The oxygen partial pressure was held at 0.20 ATA. Chamber temperature was maintained at 30°C to offset the cooling effect of helium. The chamber was decompressed daily (1 ATA per 5 min.) in order to replace the diet. Daily compression and decompression did not alter the behavior of lab chow fed mice. Control mice (n=7) were run in a separate study. They received ethanol containing liquid diet, but were housed in 1 ATA air at 21°C during acquisition. All mice were scored daily and at the onset of withdrawal for their degree of intoxication. On the test day, the experimental group was decompressed before removing the ethanol diet at 0200 hours. A 20 µl blood sample was taken from the ophthalmic venous plexus. Each mouse was then individually housed in air (1 ATA) at 25°C and rated hourly under "blind" conditions for signs of withdrawal. Hyperbaric treatment during acquisition significantly reduced the signs of physical dependence compared to controls. The experimental group did not begin to show signs of withdrawal until two hours after controls and had significantly less withdrawal symptoms at 7 out of 9 hourly ratings compared to controls. Maximum withdrawal scores were approximately 50% higher in controls compared to the hyperbaric treated mice. There were no significant differences between experimental and control groups in the intoxication scores obtained during acquisition or at the initiation of withdrawal, nor were there differences between groups in pre-withdrawal blood ethanol concentrations. Future studies must match blood ethanol concentrations during acquisition and other variables in the control and experimental groups before definitive conclusions can be drawn. But, these findings extend previous work to suggest that hyperbaric treatment concurrent with ethanol consumption attenuates the development of ethanol-induced physical dependence in mice. Taken with previous reports, these results support the hypothesis that hyperbaric treatment directly antagonizes ethanol at the membrane level. (Supported by R01 AA03972 and AA05234, NIAAA)

357.9 INFLUENCES OF AMBIENT TEMPERATURE ON THE DEVELOPMENT OF TOLERANCE TO ETHANOL INDUCED-HYPOTHERMIA. A. D. Lê*, J.M. Khanna and H. Kalant* (SPON: M. A. Linseman). Dept. of Pharmacology, Univ. of Toronto, and Addiction Res. Foundation of Ontario, Toronto, Canada M5S 1A8.

A study was conducted to evaluate whether the experience of hypothermia during ethanol treatment is of importance for the development of tolerance to its hypothermic effect. Three groups of male Wistar rats (n=10) were treated daily with ethanol (5 g/kg, p.o.) for 28 days. Group I received ethanol treatment at 4°C ambient temperature which enhances the hypothermic effect of ethanol. Group II received ethanol treatment at 36°C temperature which offset ethanol-induced hypothermia. The remaining group received ethanol treatment at normal room temperature of 22°C. An additional group received daily equicaloric sucrose treatment at room temperature. Tolerance development was monitored at 5 day intervals with a test dose of 3 g/kg, i.p., at room temperature. Tolerance to the hypothermic effect of ethanol developed rapidly when ethanol treatment was carried out at 4°C. On the other hand, rats receiving ethanol treatment at 36°C acquired tolerance much more slowly, but achieved the same level of tolerance as other groups by day 25. On day 29, when testing was carried out at 4°C, similar levels of tolerance were also observed among all ethanol treated groups. After tolerance had developed maximally, it was retained equally, whether the ethanol was now administered at 36°C or at 22°C. These results support our notion that variation in functional demand during drug exposure can modulate the rate of change in the basic physiological mechanism underlying tolerance.

357.10 THE METHOD OF CHRONIC ETHANOL ADMINISTRATION AS A FACTOR IN TOLERANCE DEVELOPMENT: LIQUID DIET VS. GAVAGE. A. Gougos*, A. D. Lê*, H. Kalant* and J. M. Khanna. Dept. of Pharmacology, Univ. of Toronto, and Addiction Res. Foundation of Ontario, Toronto, Canada M5S 1A8.

Chronic ethanol treatment by gavage (intubation), while producing tolerance to ethanol (EtOH), confers little or no cross-tolerance to hypnosis, hypothermia and motor-impairment induced by pentobarbital (PB). However, when EtOH is administered in the form of a liquid diet, cross-tolerance to the hypnotic effect of PB is readily seen. This study was designed to explain the difference between the effects of administration by the two routes.

Two groups of male Sprague-Dawley rats received EtOH chronically, either in the liquid diet form or by gavage, to a maximum of 8-10 g/kg/day. Two corresponding control groups received equicaloric sucrose. Both metabolic tolerance and functional tolerance to the hypothermic and motor-impairing effects of EtOH (2.6 g/kg, i.p.), were demonstrated in both the liquid diet and gavage groups. However, only the liquid diet treatment conferred cross-tolerance to PB hypothermia (26 mg/kg, i.p.). Blood PB levels measured at 7.5-90 min following injection were consistently lower in the EtOH-receiving group than in controls, and the disappearance rate was higher. This indicates that the observed cross-tolerance to PB following EtOH liquid diet treatment is largely due to dispositional factors.

In contrast, the gavage group showed no significant difference in blood PB levels, yet the EtOH gavage group had a marginally lower hypothermic response than its control ($0.1>p>0.05$). Thus, there appears to be a small component of functional cross-tolerance to PB which is insignificant compared to the degree of EtOH tolerance. Both gavage groups showed markedly higher blood PB levels than the liquid diet groups after the same test dose, indicative of a major difference in volume of distribution. These findings demonstrate a clear difference between the effects of the two alcohol regimens on the pharmacokinetics of PB, which may explain the variability in extent of cross-tolerance reported in the literature.

ALCOHOL AND BARBITURATES: BIOCHEMISTRY

358.1 CONTINUOUS AMPHETAMINE ENHANCES ETHANOL CONSUMPTION: ROLE OF CNS CATECHOLAMINES. A.D. Levy* and G. Ellison (SPON: A. Yuwiler), Dept. of Psychology, UCLA, Los Angeles, CA 90024.

We have previously demonstrated that amphetamine, if administered continuously at low-levels via subcutaneously implanted silicone pellets (see Neilson, E. et al. Comm. Psychopharm., 1980; 4: 17), selectively enhances consumption of a 10 % v/v ethanol (EtOH) solution (Pottoff, A. et al. Psychopharm., 1982; 77: 242). To determine if this effect is mediated by central nervous system (CNS) catecholamines, rats were given intraventricular injections of 6-hydroxydopamine (6-OHDA) for dopamine (DA) and norepinephrine (NE) depletion, or 6-OHDA preceded by i.p. injection of 25 mg/kg desipramine (DMI) to protect noradrenergic neurons, prior to amphetamine administration.

Baseline EtOH and water measurements were made at 3 day intervals for 15 days prior to intraventricular injections. Six groups of rats (N=7) received either 6-OHDA (2 x 250 µg), 6- OHDA + DMI, or vehicle, followed 3 days later by subcutaneous pellet implantation for continuous administration of either amphetamine of vehicle. Following pellet implantation access to EtOH was withdrawn for 6 days to prevent conditioned taste aversions. EtOH was then returned and fluid measurements resumed at 3 day intervals. Thirty days after implantation pellets were removed and access to EtOH was withdrawn. Animals were sacrificed 3 weeks later and brain regions were removed and assayed by liquid chromatography for DA and NE levels to determine the extent of the lesions.

There were no differences between groups for baseline EtOH or for baseline water intake. Continuous amphetamine administration enhanced EtOH consumption from baseline in nonlesioned rats. Amphetamine administration produced a similar increase of EtOH intake from prelesion baseline in animals receiving 6-OHDA + DMI, but this increase was prevented in the 6-OHDA - amphetamine group. EtOH consumption in control pellet animals was higher in the 6-OHDA + DMI group compared to nonlesioned rats.

The changes of EtOH and water intake following lesions and amphetamine administration will be discussed in terms of the relative roles of central DA and NE in the mediation of continuous amphetamine's enhancement of EtOH consumption.

358.2 POSSIBLE METABOLIC MODULATORS OF VOLUNTARY ETHANOL INTAKE. S. M. Sokaransky*, C. M. G. Aragon*, Z. Amit, (SPON: R. Clavier). Dept. of Psych., Center for Studies in Behavioural Neural Biology, Concordia University, 1455 de Maisonneuve, Montreal, Quebec, Canada H3G 1M8.

The relationship between the capacity to effectively metabolize ethanol and voluntary intake was examined in male Long Evans rats. Principally the rates of activity of enzymes involved in the formation and elimination of ethanols major metabolite-acetaldehyde, was compared to daily intake. The sub-cellular distribution of aldehyde dehydrogenase (ALDH) in brain was observed to consist of a mitochondrial and a microsomal form, with approximately 60% of total recovered activity existing in the mitochondria and 40% in microsomes. In addition, it was observed that some differences existed between these two sub-cellular forms of ALDH with respect to substrate specificity. Correlations computed between fractional activity and ethanol intake revealed significant correlations between cerebral mitochondrial activity and intake ($r=.679$, $p<0.05$), and between cerebral microsomal activity and intake ($r=.647$, $p<0.05$). The activity of cerebral catalase, an enzyme suggested to be involved in formation of acetaldehyde in brain was compared to volunatary consumption levels of ethanol. A significant correlation was observed between the activity of catalase and voluntary intake levels ($r=.570$, $p<0.05$). As the two enzymes under study are involved in the formation and elimination of acetaldehyde, a substance implicated in the psychopharmacological effects of ethanol, then perhaps these findings may represent a metabolic regulatory system for ethanol consumption.

358.3 ETHANOL BLOCKS THE CONVERSION OF 1,4 BUTANEDIOL TO GAMMA-HYDROXYBUTYRIC ACID IN VIVO. F. Poldrugo and O. C. Snead. Department of Pharmacology, Pediatrics and The Neuroscience Program. University of Alabama in Birmingham School of Medicine, Birmingham, Alabama 35233.

1,4 butanediol (1,4 BD) does not produce behavioral changes analogous to intoxicating doses of other alcohols (McCreery et al, Neuropharmacology 17, 451, 1978). The unique behavioral effects of this compound have been attributed to its conversion to γ-hydroxybutyric acid (GHB) (Sprince et al, Life Sci. 5,2041, 1966). The object of these experiments was to further characterize the behavioral and EEG changes produced by 1,4 BD, in terms of their relation to GHB, and their interaction with ethanol (ETOH).

A dose of 1 gm/kg 1,4 BD administered to rats produced behavioral and electroencephalographic (EEG) changes analogous to the effects that followed a dose of 200 mg/kg γ-butyrolactone (GBL) which is a prodrug for GHB, being converted to the latter by a circulating lactonase (Roth et al, Int. J. Neuropharmacol. 5, 421, 1966). The changes seen with 1,4 BD correlated with a marked increase in concentration of GHB in brain and liver consistently observed after 1,4 BD administration. ETOH pretreatment blocked the EEG and behavioral effects of 1,4 BD as well as the conversion of 1,4 BD to GHB.

The interaction of 1,4 BD and ETOH was also assessed, both during acute ETOH administration and during ETOH withdrawal. 1,4 BD potentiated the behavioral effect of acute ETOH administration and antagonized withdrawal tremors, but had no effect on blood ethanol levels.

The results suggest an in vivo competition for alcohol dehydrogenase between ETOH and 1,4 BD. The reported ability of 1,4 BD to potentiate the effects of an acute dose of ETOH and to antagonize ETOH withdrawal without concomitant change in blood ETOH level (BEL) may be an effect of the diol molecule itself.

Hence 1,4 BD may have two types of pharmacologic actions, one attributable to its conversion to GHB and the other an inherent property of 1,4 BD itself.

358.4 PHYSICAL PROPERTIES OF BRAIN MEMBRANES FROM ETHANOL TOLERANT-DEPENDENT MICE. R.A. Harris, M.A. Mitchell* and R.J. Hitzemann. V.A. Hospital and Dept. Pharmacol., Univ. of Missouri Sch. Med., Columbia, MO 65212 and Depts. Psychiatry and Pharmacol., Univ. Cincinnati Sch. Med., Cincinnati, OH 45267.

Ethanol tolerance and dependence may be consequences of homeoviscous adaptation of brain membranes. This mechanism requires that chronic ethanol ingestion increase the rigidity of brain membranes and decrease their sensitivity to the disordering effects of ethanol, resulting in a membrane which functions normally in the presence of ethanol, but abnormally in the absence of the drug. To test this hypothesis, we used fluorescent probes to compare the physical properties of membranes from control and ethanol-treated animals. Male DBA/2 mice were given a diet containing 5% v/v ethanol in Carnation Slender for 7 days. Controls were pair-fed an isocaloric sucrose diet. After withdrawal of the diets, dependence was evidenced by convulsions on handling (score at 7 hrs=3.1). Tolerance was demonstrated by a 70% increase ($p<0.01$) in the ethanol ED_{50} for loss of righting reflex, and a 20% increase ($p<0.01$) in the brain ethanol concentration at regaining of righting reflex (as compared to controls). SPM (synaptic plasma membranes) were prepared from brain and the fluorescent probes DPH (diphenylhexatriene), TMA-DPH (trimethylammonium-DPH), cis-P (parinarate) and trans-P were incorporated (Harris and Schroeder, J. Pharmacol. Exp. Ther. 223:424, 1982). The baseline fluorescence polarization of DPH, cis-P and trans-P (probes of the lower portions of acyl groups) was higher in SPM from ethanol tolerant-dependent mice than in controls. The fluorescence polarization of TMA-DPH (a probe of the upper portions of acyl groups) was not affected by chronic alcohol treatment. In vitro exposure to ethanol (50 to 300 mM) decreased the polarization of DPH in control SPM but this effect of ethanol was attenuated in SPM from ethanol tolerant-dependent mice. This effect of chronic ethanol exposure was more pronounced in SPM than in microsomal or mitochondrial fractions. In contrast to intact SPM, the lipids extracted from SPM of ethanol tolerant-dependent mice did not differ from controls in baseline polarization of DPH fluorescence or in sensitivity to in vitro ethanol exposure. These results demonstrate that chronic ethanol treatment increased the rigidity of the hydrophobic core of synaptic membranes and reduced their sensitivity to the membrane disordering effects of ethanol, consistent with homeoviscous adaptation. The data for extracted lipids, together with those in the accompanying abstract (Hitzemann et al.), suggest that the changes in membrane physical properties are not due to changes in lipid composition, but may be due to changes in lipid arrangement (asymmetry, domains) or lipid-protein interactions. Supported by the Veteran Administration and USPHS DA02855.

358.5 LIPID COMPOSITION OF BRAIN MEMBRANES FROM ETHANOL TOLERANT-DEPENDENT MICE. R.J. Hitzemann, R.A. Harris and M.A. Mitchell,* Departments Psychiatry and Pharmacology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267 and V.A. Hospital and Department of Pharmacology, University of Missouri School of Medicine, Columbia, Missouri 65212.

The results described in the accompanying abstract (Harris et al.) demonstrate that chronic ethanol administration increases the hydrophobic core of synaptic membranes and reduces their sensitivity to the membrane disordering effects of ethanol. The mechanisms underlying this apparent homeoviscous adaptation are unknown but by analogy with similar processes occurring during temperature adaptation may involve the membrane lipids. In the present study we have examined the lipid content and composition of synaptic membranes prepared from ethanol-treated (E) and control (C) animals. Male DBA/2 mice were given a diet containing 5% v/v ethanol in Carnation Slender for 7 days. Controls were pair-fed an isocaloric sucrose diet. Tolerance and dependence in the E animals is described in the accompanying abstract (Harris et al.). Synaptic membranes were prepared and their lipid content was analyzed as described elsewhere (Hitzemann and Johnson, Neurochem. Res. 8:121, 1983). All data = mean ± S.D.; N=6. N.S. = no significant ($p > 0.05$) difference.

The cholesterol/lipid P molar ratio was 0.58 + 0.12 (E) and 0.72 + 0.08 (C) (N.S.). The ratio of u mole lipid P/mg protein was 0.94 ± 0.30 (E) and 0.86 + 0.26 (C) (N.S.). No significant difference was found in the composition of the major phospholipids. The acyl-linked fatty acid composition of the various phospholipids was examined. Forty-six categories were compared. Only two significant ($p < 0.05$) differences were found. First, the amount of 16:0 in sphingomyelin was greater in the E animals (8.1 ± 8.8% vs. 0.8 + 0.8%). Secondly, the amount of 22:6 (W-3) in phosphatidylserine was less in the E animals (18.8 + 5.7% vs. 27.9 + 4.6%). The ratio of ganglioside (n mol NANA)/lipid P (n mol) was 0.13 ± 0.03 (E) and 0.12 + 0.02 (C) (N.S.). Furthermore, there was no significant difference in the percent distribution of 11 different gangliosides as determined by scanning densitometry. Overall, these results suggest that the changes in synaptic membrane biophysical properties accompanying ethanol tolerance-dependence development are not associated with significant changes in lipid content or composition. Supported by grants MH-37377, DA-02855 and the Veterans Administration).

358.6 IMMUNOCYTOCHEMISTRY OF HYPOTHALAMIC AND AMYGDALOID SUBSTANCE P: EFFECTS OF CASTRATION AND ETHANOL. W. Les Dees* and Gerald P. Kozlowski. Department of Physiology, University of Texas Health Science Center at Dallas, Southwestern Medical School, Dallas, TX 75235.

Recently, it has been shown that ethanol (ETOH) depresses hypothalamic luteinizing hormone releasing hormone (LHRH) release (Dees et al., Biol. Repro. June, 1983). Whether ETOH is acting directly upon LHRH neurons or indirectly by altering the synthesis or release of other substances which may in turn affect LHRH release is not known. Since substance P (SP) is present in high quantities in central and medial amygdaloid nuclei (CM-AM), and since this region produces facilitatory effects on gonadotropin secretion, it seems possible that SP could be involved in mediating LHRH release. The anatomic connections known to exist between the amygdala and hypothalamic-preoptic regions, along with the suggestion that SP terminals are in contact with LHRH cell bodies (Hoffman et al., Neurosci. Abstr. 8: 109, 1982) further supports this hypothesis. Physiologic data also indicates an interrelationship between these two peptides; however, the exact nature, of this interaction has thus far been conflicting (Kerdelhué et al., Endo. Abstr., 186, 1978; Vijayan and McCann, Endocrinology 105: 64, 1979). To further analyze the possible interrelationship which may exist between the SP and LHRH systems, we felt it important to determine if SP immunoreactivity in the brain would react to castration and ETOH in the same manner as LHRH. Thus, these studies were designed to determine what effects these parameters would have on SP immunoreactivity in the hypothalamus and CM-AM. Paraffin sections of rat brains were stained for SP using the PAP method and a specific antserum to SP (Kozlowski #317). Obvious differences visualized immunocytochemically between saline-treated intact and castrated rats indicated that the SP content of the CM-AM was markedly depleted after castration. Conversely, this castration-induced reduction in SP fibers was diminished by ETOH treatments (1.25 g/kg, every 6 hr. for 8 days). ETOH was also effective in increasing the number of SP fibers in the CM-AM in intact animals. Similar results were seen for specific regions of the hypothalamus, although they were less pronounced than that visualized in the CM-AM. Thus, these data indicate that both castration and ETOH administration affects hypothalamic and amygdaloid content of SP. Since these effects on SP are similar to those previously shown for LHRH, it is possible that ETOH diminishes the release of SP, and that SP is involved in LHRH release. (Supported by AA 06014-01).

358.7 ETHANOL'S EFFECTS ON CORTICAL ADENYLATE CYCLASE ACTIVITY. T. Saito,[1]* J.M. Lee[1] and B. Tabakoff.[1,2] [1]Alcohol and Drug Abuse Research and Training Program , Department of Physiology and Biophysics, University of Illinois at Chicago, Health Sciences Center and [2]Westside VA Medical Center, Chicago, IL 60612.

Although high concentrations of ethanol have previously been shown to increase adenylate cyclase (AC) activity in membrane preparations from several tissues, we have recently demonstrated that physiologically relevant concentrations of ethanol activate striatal AC only in the presence of guanine nucleotides (GTP or Gpp(NH)p). In the current studies, we examined beta-adrenergic receptor-coupled, cortical, AC activity to expand our understanding of ethanol's effects on brain AC systems.

AC activity in membrane preparations of cerebral cortex of C57Bl mice was increased by addition of GTP or Gpp(NH)p to incubation mixtures. Addition of isproterenol or norepinephrine further increased enzyme activity. Propranolol (1 μM), but not phentolamine (10 μM), was able to block the isoproterenol stimulation of AC activity. Ethanol, in concentrations up to 500 mM, had little effect on AC activity in washed membrane preparations of mouse cortex. However, after addition of Gpp(NH)p (10 μM) to incubation mixtures, ethanol stimulated AC activity significantly beyond the level of activity produced by the addition of Gpp(NH)p alone. Addition of 50 mM ethanol resulted in a 40% increase in AC activity. The slope of the dose-response curve for ethanol stimulation of AC activity in the presence of Gpp(NH)p was biphasic, but the addition of isoproterenol eliminated the initial rapid increase in AC activity produced by low concentrations of ethanol. The dose-response curve for ethanol stimulation of AC activity was linear in the presence of both Gpp(NH)p and isoproterenol.

Although the presence of ethanol did not alter the "K_m" for Gpp(NH)p activation of AC, the time course of activation of cortical AC activity by Gpp(NH)p (hysteretic effect) was altered by ethanol. The $T_{\frac{1}{2}}$ (23°C) for activation was 8.7 minutes and 10.7 minutes, respectively, in the presence and absence of ethanol (250 mM). Ethanol did not alter the K_m of the AC system for Mg-ATP.

Experiments using systems in which the G/F protein was preactivated by Gpp(NH)p demonstrated that ethanol could still increase AC activity, but only in a linear dose-response manner.

We postulate two sites of action for ethanol in this system: one site involves the interaction of beta-adrenergic agonist-receptor complex with the G/F protein, and the other sites involves the interaction of the G/F protein with the catalytic unit of AC.

[Supported in part by NIAAA, NIDA and VA Medical Research Service]

358.8 ETHANOL DECREASES SYNAPTOSOMAL CALCIUM-DEPENDENT NEUROTRANSMITTER RELEASE. R. Strong*, J. Walsh* and W. G. Wood (SPON: J. C. Schoolar). GRECC, VA Med. Ctr., and St. Louis Univ. Sch. of Med., St. Louis, MO 63125

Most investigators agree that ethanol (ETOH) acts at synapses to produce its effects. Both pre- and post-synaptic effects have been described for anesthetic concentrations of ETOH. ETOH has been reported to either depress or increase depolarization-dependent Ca influx into synaptosomal preparations. In that Ca influx provides the trigger for transmitter secretion, an alteration of depolarization-triggered Ca entry by ETOH might be sufficient to result in enhancement or depression of transmitter release. In the present series of experiments, we investigated the effects of ETOH (75-500 mM) upon Ca-dependent secretion of norepinephrine (NE) and γ-aminobutyric acid (GABA) from synaptosomes isolated from C57Bl/6J mouse cortex. Isolated synaptosomes provide an ideal preparation to investigate drug effects in that the procedure eliminates direct interneuronal interractions and minimizes diffusional barriers allowing accurate control of extracellular fluid composition. Synaptosomes were pre-labeled with either ^{14}C-GABA or ^{3}H-NE, pre-incubated with different concentrations of ETOH, and then washed with HEPES-Ringers buffer every 40 seconds in a negative pressure filtration system. Depolarization agents (KCl and veratrine) were added during the seventh-tenth washes (stimulation period) to facilitate Ca-dependent release. Ethanol was also added during stimulation and washout. KCl (50 mM) or veratrine (100 μM) increased release of GABA or NE 2- to 3-fold above baseline conditions (sixth washout). Ethanol in concentrations from 150-500 mM significantly depressed release above baseline but had no effect on baseline release itself. Ethanol's depressant effect was greater when veratrine was the depolarizing agent suggesting a selective effect of ethanol on Ca channels associated with the Na ionophore. The present results are consistent with the notion that ethanol alters neurotransmitter release by decreasing Ca entry into the nerve terminal. Experiments are underway to determine whether ethanol is acting specifically on one of the ionophores, i.e., Ca, Na, K, or on all of them equally.

Supported by the Medical Research Service of the Veterans Administration and the Geriatric Research, Education and Clinical Center of the VA Medical Center, St. Louis, Missouri.

358.9 INTRACELLULAR CALCIUM-RELEASE BY ETHANOL IN RAT BRAIN SYNAPTOSOMES. U. Pande*, H. C. Pant, and F. F. Weight. Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

The effect of ethanol on calcium metabolism in synaptosomes was studied employing the Ca^{2+}-indicator dye Arsenazo III. Synaptosomes from rat brain were suspended in isotonic medium (pH=7.32) containing 20mM Tris-HCl,132mM NaCl,5mM KCl,1mM EGTA and 9μM Az III. Calcium titration experiments were performed on: (1) the medium; (2) a suspension of synaptosomes (30μg protein/ml) in the medium (control); and (3) suspensions of synaptosomes to which 10-100mM ethanol was added. In these experiments, relative changes in the absorbance of the Ca-Az complex at 650nm were used as an indicator of changes in synaptosomal calcium. The addition of 10-100mM ethanol to the synaptosomal suspension increased the absorbance of the Ca-Az complex at 650 nm (A_{650}). Increasing concentrations of ethanol resulted in larger increases in A_{650}. Titration experiments performed with the addition of 10-100 mM ethanol to the medium containing Az III but no synaptosomes resulted in no change in A_{650}. This indicates that ethanol does not interact directly with Az III to cause a change in A_{650}. If ethanol was replaced by equal volumes of isotonic buffer or sucrose in the synaptosomal suspension, no change in A_{650} was observed. These results show that ethanol releases calcium from synaptosomes and that the release of calcium is not due to osmotic changes. If 20-100μM theophylline was added to the synaptosomal suspension, results similar to the effect of ethanol were obtained. Ethanol and theophylline had no effect on A_{650} in lysed synaptosomal preparations. In order to test whether an intracellular release of calcium might be involved in the release of calcium from synaptosomes, the experiments were repeated in the presence of dantrolene sodium (DaNa). This drug inhibits the intracellular release of Ca^{2+} from sarcoplasmic reticulum of skeletal muscle (Desmedt & Hainaut, Biochem. Pharmacol. 28, 957-964, 1979). The addition of ethanol to the synaptosomal suspension did not change A_{650} when 50μM DaNa was present in the medium. These observations suggest that 10-100 mM ethanol can release calcium from intracellular stores in rat brain synaptosomes.

358.10 THE EFFECT OF GABA OR BENZODIAZEPINE RECEPTOR ANTAGONISTS ON THE ANTICONFLICT PROPERTIES OF DIAZEPAM OR ETHANOL: S. Liljequist* and J.A. Engel. Dept of Pharmacology, University of Göteborg, Box 33031, S-400 33 Göteborg, Sweden.

The effects of picrotoxin, bicuculline, or Ro 15-1788 on the anticonflict action of diazepam or ethanol, were studied in rats using a modified Vogel's conflict test procedure. Animals were deprived of water for 48 h, whereafter they were placed in a test box equipped with a grid floor and a drinking bottle containing 5.5 % (w/v) of glucose. Upon finding the drinking spout the animals were allowed to drink of the glucose solution for 30 s after which time an electric shock (with a current of 0.16 mA and given for 2 s every 3 s) was given. Thereafter every subsequent attempt of the animals to drink was punished with an electric shock during the 10 min experimental session, and the number of shocks taken by the animals was recorded.

The benzodiazepine receptor antagonist Ro 15-1788 dose-dependently antagonized the antipunishment effects of diazepam (2.5 mg/kg, i.p.), whereas various doses of drugs affecting central GABAergic mechanisms like bicuculline or picrotoxin did not interfere with diazepam's effect in this test situation. The anticonflict action of ethanol (2 g/kg, i.p.) was antagonized by picrotoxin (1.0 mg/kg, i.p.), whereas both bicuculline and Ro 15-1788 were without effect on the increased punished responding produced by ethanol. These data support the concept that the anticonflict properties of diazepam are not mediated via direct activation of GABAergic receptor mechanisms. On the other hand, these data lend further evidence to the contention that at least some of the effects of ethanol are mediated through an enhancement of central GABAergic activity, possibly through an activation of a picrotoxin-sensitive site in the GABA-benzodiazepine-receptor-ionophore complex.

This study was supported by the Swedish Medical Research Council (4247), Torsten och Ragnar Söderbergs Stiftelse, Magnus Bergvalls Stiftelse, Stiftelsen Sigurd och Elsa Goljes Minne, and the Medical Research Council of the Swedish Life Insurance Companies.

358.11 **EFFECTS OF CHRONIC ETHANOL INTAKE ON CORTICAL AND HIPPOCAMPAL BENZODIAZEPAM RECEPTOR BINDING IN FEMALE RATS.** **J.L. Steinberg, C. Co*, C.S. Sebastian* and S.I. Dworkin. Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.**

Chronic ethanol administration has been demonstrated to alter both neurotransmitter receptor binding and the physical properties of membranes. Several studies have indicated that the species, dose, sex and duration of exposure to ethanol are important determinants of a drug's neurochemical effects. The present study reports the changes in both cortical and hippocampal benzodiazepine binding after 50 days of exposure to an ethanol diet.

Eight female F-344 old rats were isolated in individual enclosures. Half of the subjects were then placed on an ethanol (95%) diet (Bio Serv), the other four subjects were fed a calorically balanced control diet (Bio Serv). The control-fed animals ingested between 2.0 to 3.4 ml/hour over a 50-day period. Over the same time period, four ethanol-fed subjects consumed less of the diet (between 1.2 to 2.4 ml/hour), and had an average ethanol intake of 7.1 g/kg/day.

Following 50 days of exposure to the diets, all eight subjects were sacrificed by decapitation and the cerebral cortex and hippocampus were isolated by dissection. Synaptic membrane preparations were made in 50 nM Tris-HCl buffer. The prepared synaptic membranes were incubated in duplicate for 20 minutes at 0°C in 1 ml of buffer containing seven different concentrations of [^{3}-H]-flunitrazepam (.12 nM - 8.0 nM)in the presence or absence of 5 uM clonazepam. A decrease in the Bmax was observed for benzodiazepine receptor binding in the cortex of the ethanol treated subjects. Receptor binding data in the hippocampus showed a decrease in the Bmax and a corresponding increase in the KD of the ethanol-treated rats. These data suggest that chronic ingestion of large doses of ethanol by female rats results in changes in benzodiazepine binding. (Funded in part by the Edward P. Stiles Trust Fund).

358.12 CHANGES IN MUSCARINIC CHOLINERGIC RECEPTORS AND CHOLINE ACETYLTRANSFERASE ACTIVITY FOLLOWING WITHDRAWAL FROM LONG-TERM ETHANOL ADMINISTRATION. E. D. Witt, C. R. Mantione, and I. Hanin. Dept. of Psychiatry, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15213.

Long-term ethanol(Et) consumption (18-73 weeks) has been shown to increase muscarinic receptor binding (^{3}H-QNB) in rat striatum and mammillary bodies either immediately, 8 days, or 4 weeks after withdrawal. In these same brain areas, choline acetyltransferase (ChAT) activity was reduced when measured immediately and 4 weeks after withdrawal (Pelham, R.W., et al., Alcoholism: Clin. Exp. Res., 4:282, 1980; Nordberg, A. and Wahlstrom, G., Life Sci., 31:277, 1979). In an attempt to further characterize the region-specific and time-dependent changes in ChAT activity and ^{3}H-QNB following chronic Et exposure, male Long-Evans black hooded rats were administered Et (6% v/v) in a liquid diet. Pair-fed controls were given the same diet except that sucrose was isocalorically substituted for Et. After 18 weeks of Et consumption, the animals were removed from their respective diets. Eight days later, ChAT activity and ^{3}H-QNB were measured at saturating substrate conditions in the striatum, hippocampus, and cortex.

As expected, 8 days after withdrawal from Et, ^{3}H-QNB in the striatum increased, but not significantly to 20% above control values (treated:403 ± 53; controls:335.6 ± 11 pmoles/gm tissue). Additionally, in our studies, ChAT activity was significantly elevated in the striatum to 52% above control values (treated: 29.7 ± 4; controls:19.6 ± .45 nmoles ACh synthesized/mg protein/hr) ($p<.01$). In the hippocampus, ^{3}H-QNB was elevated, but again not significantly, to 10% above pair-fed controls (treated:370 ± 23; controls:336.3 ± 9 pmoles/gm tissue), while no change occurred in ChAT activity. There was no significant effect on ChAT or ^{3}H-QNB in the cortex.

Our observed increase in striatal ^{3}H-QNB may be a compensatory postsynaptic response to long-term Et consumption which, as reported by others, persists for several weeks postwithdrawal. However, the increase in ChAT activity which we observed suggests that there also is a presynaptic adaptation 8 days after Et withdrawal, in the direction of increasing cholinergic activity. This coincides with a graded rise in high affinity choline transport, reported to occur in Et-dependent rats after withdrawal (Hunt, W.A. and Majchrowicz, E., Drug and Alcohol Depend., 4:245, 1979). These combined findings implicate suppression of cholinergic activity during chronic Et consumption followed by both presynaptic and postsynaptic adaptive changes upon withdrawal from Et. Supported by Alcoholic Beverage Medical Research Foundation and NIMH Grant #26320.

358.13 ADENYLATE CYCLASE IN THE HUMAN PLATELET: MODULATION OF ACTIVITY BY PROSTAGLANDINS, EPINEPHRINE AND ETHANOL. J.M. Lee,[1] T. Saito[1]* and B. Tabakoff.[1,2] [1]Alcohol and Drug Abuse Research and Training Program and Department of Physiology and Biophysics, University of Illinois at Chicago, Health Sciences Center and [2]Westside VA Medical Center, Chicago, IL 60612.

We have, in the past, characterized the effects of ethanol on brain adenylate cyclase (AC) activity which is stimulated by addition of catecholamines to our assay systems. Ethanol was demonstrated to potentiate the effects of both guanine nucleotides (GTP, Gpp(NH)p) and catecholamines on the AC preparations. In addition to the catecholamine receptor systems which stimulate AC activity (e.g., D_1 and beta-adrenergic), there are present, in brain and other tissues, catecholamine receptors which act to inhibit AC activity (e.g., $alpha_2$ receptors). In attempts to expand our knowledge of ethanol's mechanism of action, we chose to examine the effects of ethanol on the AC activity in a model system, the human platelet.

Under our assay conditions (10 mM Mg^{++}, 0.25 mM ATP), both GTP and Gpp(NH)p slightly inhibited AC activity. Prostaglandin-E_1 (PGE_1) alone stimulated AC activity. The interaction of PGE_1 and GTP on platelet AC activity were dependent on the concentration of sodium present in the assay system. In addition, epinephrine (0.1-100 μM) diminished the PGE_1 (1 μM)-stimulated AC activity by a maximum of 30%. This effect of epinephrine required the addition of both sodium and guanine nucleotides to our assay system. Although ethanol (250 mM) produced a small, but significant (16 ± 4%) increase in "basal" AC activity, ethanol did not alter the effect of either PGE_1 or epinephrine in the presence or absence of guanine nucleotides. On the other hand, the CsF-mediated activation of platelet AC was significantly enhanced by ethanol. In the presence of 250 mM ethanol and CsF (10 mM) AC activity was 57 pmol/min/mg protein, while AC activity with CsF alone was 46 pmol/min/mg protein. The effects of ethanol on CsF-stimulated AC activity did not involve a change in the K_m of the enzyme for ATP.

Our results indicate a substantial difference in ethanol's effects on catecholamine receptor systems coupled in a stimulatory versus inhibitory manner to AC. Stimulation of AC through the N_s coupling protein is potentiated by ethanol, but inhibition of AC through the N_i coupling protein is little affected by ethanol.

[Supported in part by NIAAA, NIDA and VA Medical Research Service.]

358.14 CHANGES IN TYROSINE HYDROXYLASE ACTIVITY IN THE ADRENAL GLAND OF MICE FOLLOWING ACUTE ETHANOL. T. French*, J. Masserano and N. Weiner (SPON: K.A. Heidenreich). Dept. Pharmacology, Univ. Colo Health Sci. Center, Denver, CO 80262.

We have been examining catecholaminergic systems in lines of mice selectively bred for a differential sensitivity to acute administration of a hypnotic dose of ethanol. These lines of mice have been designated long sleep (LS) and short sleep (SS). In the present study tyrosine hydroxylase (TH) activity and catecholamine (CA) levels were measured in the adrenal glands of LS and SS mice following a 4.0 g/kg dose of ethanol administered intraperitoneally as a 20% w/v solution. At 0, 10, 25, and 125 minutes after ethanol the adrenal glands were surgically removed under halothane anesthesia. Basal levels of TH activity, epinephrine (EPI), and norepinephrine (NE) were significantly higher in the adrenal glands of LS mice by approximately 70%, 20%, and 30%, respectively. Ten minutes following ethanol administration, LS adrenal gland TH activity is decreased by approximately 30% while SS adrenal gland TH activity is increased approximately 70%. At 25 and 125 min following ethanol, SS adrenal gland TH activity is no longer increased, but LS TH activity is still significantly decreased. The corresponding adrenal CA levels were unchanged in the LS mice at all times measured except at 125 min when both EPI and NE were decreased about 25-30%. In the SS mice, adrenal EPI levels were increased at 10 min after ethanol, but at all other times NE and EPI were not different from control. Prior administration of chlorisondamine (15 mg/kg, i.p.) had no effect on the ethanol induced changes in adrenal TH activity in the LS mice at 10 min. However, in the SS mice the increase in TH activity seen 10 min after ethanol is essentially blocked by pretreatment with chlorisondamine. A hypnotic dose of pentobarbital (65 mg/kg, i.p.) had no effect on adrenal gland TH activity of either LS or SS mice when measured at 0, 15, 60, and 80 min after injection. These data are consistent with a specific, transient stimulation of the adrenal medulla of SS mice consequent to ethanol administration, that may be mediated via the central nervous system. This response to ethanol is apparently absent in the LS mice. Supported by USPHS grants NS 07927 and AA 03527.

358.15 ENDOGENOUS BRAIN STEROIDS: EFFECT OF ACUTE ETHANOL INGESTION. Colette Corpechot*, William J. Shoemaker, Floyd E. Bloom and Etienne-Emile Baulieu*. The Salk Institute, La Jolla, CA 92037 and U33 INSERM, Bicetre, France.

Dehydroepiandrosterone (DHA) and pregnenolone (P) have been measured using radioimmunoassays in male rat brain. The brain levels of these steroids are independent of the presence of the adrenal glands or the testes. The distribution within the brain varies 5-fold from region to region, is greater than the concentration present in the plasma, and is unchanged when brains are perfused free of blood before dissection. We now report that oral gavage with ethanol (2 gm/kg body weight) profoundly lowers levels of both steroid within one hour. Male Sprague-Dawley rats (about 200 g) were orally gavaged with a 20% (w/v) ethanol solution at a dose of 2 g/kg. Controls were given equal volumes of water. Animals were sacrificed 1 hour later and the brain dissected for steroid assay as previously described (PNAS 78: 4704, 1981). At that time the blood alcohol level averaged 76 mg/dl. The results from two regions are given below (ng/gm tissue).

	olfactory bulb		hypothalamus	
	P	DHA	P	DHA
control (n=10)	24.2 ± 2.34	9.36 ± 0.69	7.16 ± 0.65	9.51 ± 0.96
ethanol (n=10)	18.0 ± 1.89	2.24 ± 1.18	4.59 ± 0.60	<.02
t-test	p<.05	p<.001	p<.01	p<.001

The lower levels of the steroids cannot be due to ethanol's interference with the extraction since recovery from the extraction, measured with trace radiolabeled DHA, was the same in control or ethanol treated tissues. The virtual absence of DHA in several samples (absolute assay limit = 10 pg) suggests that ethanol may interfere with the enzyme converting P to DHA, since P is the precursor. The rapidity of the effect could suggest that these steroids maintain a rapid metabolic turnover; studies are in progress to determine the time-course of recovery from ethanol, as well as the effect of chronic ethanol treatment. (Supported by NIAAA 03504.)

358.16 Comparison of the Effects of Ethanol and 2-Chloroadenosine on the High Affinity Choline Uptake in Rat Brain Using Improved Methodology. P. Szot* and T.F. Murray. (Spon: H.L. Komiskey), Washington State University, College of Pharmacy, Pullman, WA 99164-6510.

The acute and chronic administration of ethanol has been reported to alter high-affinity choline uptake (HACU) in rat brain (Hunt et al., J. Pharmacol. Exp. Ther., 210: 259, 1979). We have further characterized the acute effects of ethanol on HACU in discrete regions of the rat brain using an improved assay methodology for measuring hemicholinium-3 (HC-3) sensitive HACU in rat brain synaptosomes. In addition, we have compared the effects of 2-chloroadenosine (2-CLA) on HACU with those of ethanol.

Following incubation with (^{3}H)-choline, aliquots of synaptosomes were layered onto 0.6 ml of 70% dibutylpthalate: 30% peanut oil mixture in 1.5 ml polypropylene centrifuge tubes. These tubes were then immediately centrifuged to achieve a rapid separation of the synaptosomes from the incubation medium. The medium and dibutylypthalate containing phases were then aspirated and the remaining pellet was solubilized. Using this methodology the HC-3 sensitive HACU was highest in striatal synaptosomes, intermediate in hippocampal synaptosomes, somewhat lower in cortical synaptosomes, and virtually absent in cerebellar synaptosomes. The acute administration of ethanol (8 g/kg, p.o.) elicited a 50% reduction in hippocampal HACU and a 16% reduction in cortical HACU. As this dose of ethanol also produces a significant hypothermic effect (mean body temp. = 36.2°C), we evaluated the role of this hypothermia in ethanol's effect on hippocampal HACU. In animals whose body temperature was maintained at 37.7°C following the 8 g/kg dose of ethanol, the ethanol-induced reduction in HACU was completely prevented. 2-CLA (10 mg/kg, i.v.), in a dose which produced a modest hypothermic effect (0.6°C decrease), also reduced HACU in the hippocampus and cortex by 33% and 43%, respectively. When 2-CLA treated animals body temperature was maintained at 37.8°C the reduction in HACU associated with this dose of 2-CLA was unaltered. Therefore, the ethanol (8 g/kg)-induced reduction in HACU does not appear to be the result of a direct effect of ethanol on cholinergic neurons, but rather a consequence of the hypothermia produced by this compound. In contrast, the 2-chloroadenosine-induced reduction in HACU appears to be independent of this compound's hypothermic effect. (This investigation was supported by funds provided by the State of Washington Initiative Measure No. 171 for medical and biological research on alcoholism.)

358.17 EFFECT OF ETHANOL ON CENTRAL CATECHOLAMINE FUNCTIONS IN RAT STRAINS SELECTED FOR DIFFERENCES IN ALCOHOL-RELATED BEHAVIORS. K. Kiianmaa*. (SPON: European Neuroscience Association). Research Laboratories, State Alcohol Monopoly, SF-00101 Helsinki 10, Finland.

Earlier work has demonstrated that different inbred strains of mice which differ in their behavioral sensitivity to ethanol also differ considerably in their catecholamine neuronal sensitivity to ethanol (Tabakoff, B. & Kiianmaa, K., Soc. Neurosci. Abs. 7, 311, 1981). Thus, genetically determined differences could exist in neurosensitivity to ethanol, and the behavioral effects of ethanol might be mediated by the action of ethanol on central catecholamine neurons. In the present study the effects of an acute dose of ethanol on central catecholamine neurons were studied in four rat strains specially selected for alcohol-related behaviors (AA and ANA for high and low alcohol consumption and AT and ANT for differential sensitivity to ethanol intoxication). The levels of dopamine, noradrenaline and DOPAC were measured in the striatum and the cerebral cortex after administration of saline or ethanol (2 or 4 g/kg ip), using high performance liquid chromatography with electrochemical detection. The rates of DOPA and DOPAC formation were monitored after inhibition of the aromatic amino acid decarboxylase with NSD-1015. In agreement with previous findings, alcohol preferring AA rats had higher levels of dopamine in the striatum than the alcohol avoiding ANA rats. Ethanol did not have any effect on the levels of dopamine or noradrenaline in either strain. However, the dose of 4 g/kg of ethanol significantly increased formation of DOPA and DOPAC in the striatum of both AA and ANA rats, but the strains did not differ in these effects of ethanol. The concentration of dopamine in the striatum of alcohol sensitive ANT rats was found to be higher than that of the alcohol non-sensitive AT rats. Ethanol did not alter the levels of either dopamine or noradrenaline in these structures. The rate of accumulation of DOPA was significantly increased in both the striatum and the cerebral cortex after ethanol (2-4 g/kg) in these two strains. The metabolism of dopamine in the striatum was accelerated, too, but, again the strains did not seem to differ in their responses to ethanol.

In agreement with previous findings ethanol was found to activate catecholamine functions in the striatum and the cerebral cortex of rats suggesting that at least some of the effects of ethanol on behavior might be mediated via the effects of ethanol on these systems. The ethanol-induced changes in catecholamine functions in the striatum and the cerebral cortex were not, however, different in the four strains and, therefore, cannot apparently explain the differences among these strains in ethanol consumption and intoxication.

358.18 ETHANOL-INDUCED ELEVATION OF PLASMA CORTICOSTERONE IN RATS WITH HIPPOCAMPAL OR LATERAL SEPTAL LESIONS, J. Brick and L. A. Pohorecky*, Center of Alcohol Studies, Rutgers University, Piscataway, New Jersey 08854

We have previously shown that ethanol attenuates stress-induced increases in plasma corticosterone under a wide range of conditions (Brick and Pohorecky, Stress and Alcohol Use, Elsevier Biomedical Press, 1983, 389-402; Brick and Pohorecky, Psychopharm. 77:81-84, 1982; Pohorecky et al. Alc. Clin. Exp. Res. 4:423-426, 1980). We interpreted these results as biochemical support for a tension reduction hypothesis of alcohol use. Virtually nothing is known, however, about the neural systems involved in this effect.

Since ethanol alters brain catecholamines, nuclei which are innervated by catecholaminergic neurons and which show neurophysiological changes in response to ethanol may be involved in the ethanol-stress interaction we have described.

To test this hypothesis, lesions were made in the lateral septum or hippocampus of male Sprague Dawley rats. Subjects were handled 3 days prior to, and every day post surgery. One week after surgery, subjects were given 2 g/kg of ethanol (20% w/v) or an equivalent volume of saline and decapitated 30 minutes later. Plasma was collected for determination of corticosterone and blood ethanol levels. Bilateral electrolytic lesions of the lateral septum, compared to sham-operated controls, did not alter levels of corticosterone after saline injection. There were no significant differences between surgery groups in the elevation in plasma levels of corticosterone by ethanol or in blood ethanol levels.

In the hippocampal study no differences were observed between sham-operated or cortical lesion groups in response to saline or ethanol; therefore, the results from these groups were combined. Bilateral aspirations of the hippocampus, compared to controls, significantly increased the corticosterone response to ethanol (117% vs 40%, respectively).

These results suggest that the hippocampus may be involved in the neural pathway upon which ethanol acts to alter plasma corticosterone levels.

Supported by USPHS grants 00045, 04238.

358.19 DIFFERENTIAL BEHAVIORAL EFFECTS OF AN ADENOSINE ANALOG (L-PHENYL-ISOPROPYLADENOSINE) IN MICE SELECTIVELY BRED FOR SENSITIVITY TO ETHANOL T.V. Dunwiddie and W.R. Proctor (SPON: J. C. Kinnamon). Dept. of Pharmacology, Univ. Col. Health Sci. Ctr., and VA Medical Center, Denver, CO 80262.

Adenosine has been shown to have a potent depressant effect on the electrophysiological activity of the central nervous system. Metabolically stable analogs of adenosine, such as L-N6-phenyl-isopropyl adenosine (L-PIA) share the depressant effects of adenosine, bind with high affinity to receptor sites in the brain, and have potent sedative actions in mice (Dunwiddie and Worth, J. Pharmacol. Exp. Ther. 220:70-76, 1982).

In the present study we have analyzed the sedative effects of L-PIA in mice selectively bred for differential sensitivity to the soporific effects of ethanol. Long sleep (LS) mice demonstrate a markedly greater sensitivity than do short sleep (SS) mice, whereas the heterogeneous stock (HS), an inbred population pooled from eight strains of mice from which the LS and SS lines were derived, show intermediate sensitivity. L-PIA was injected i.p. and the activity levels of the mice were monitored 30 minutes later. At a dose of 0.1 mg/kg L-PIA, exploratory activity of LS animals was depressed by 82% compared to control animals, whereas SS mice were depressed by only 16%. The dose of L-PIA calculated to elicit a 50% reduction in activity for the three groups were: LS = 0.07 mg/kg, SS = 0.29 mg/kg, and HS = 0.10 mg/kg.

Differential sensitivity to the hypothermic effects of L-PIA were also observed. The decrease in rectal temperature for LS mice (6.0 ± 0.4 °C) was significantly greater than the decrease seen in SS mice (1.8 ± 0.5 °C). The hypothermic effect of L-PIA was significantly greater in both lines of mice than that of ethanol at doses which elicited equivalent behavioral responses. In order to determine the relationship between the hypothermic and behavioral responses, mice were tested in a warm (30 °C) environment. Under these conditions, differential behavioral sensitivity was still maintained in the absence of significant differences in rectal temperature. Subsequent biochemical analyses of brain levels of 3H-L-PIA 30 minutes after injection showed no significant differences.

Thus, it appears that mice selectively bred for differential sensitivity to the soporofic effects of ethanol (LS and SS mice), demonstrate a differential behavioral as well as physiological responsiveness to an analog of adenosine, L-PIA. This effect does not appear to reflect differential brain levels of L-PIA in the two lines of mice.

This research was supported by grants VA 394463116-01 and DA 02702 to T.V.D., and grant DA 07043-07 to W.R.P.

358.20 THE EFFECT OF ACUTE BARBITAL ADMINISTRATION ON CEREBELLAR cGMP LEVELS IN CONTROL AND BARBITAL-DEPENDENT RATS. S. Lane* and W.W. Morgan. Dept. of Anatomy, Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX 78284

Chronic administration of barbiturates has been shown to result in dependence in both man and laboratory animals. The abrupt withdrawal of these drugs produces a serious, potentially life threatening withdrawal syndrome. The biochemical mechanism(s) mediating barbiturate dependence and withdrawal symptoms have not been clearly identified. Recently in our lab it has been shown that the acute administration of pentobarbital, phenobarbital or barbital in sub-anesthetic dosages results in a dramatic reduction of cyclic guanosine monophosphate (cGMP) levels in the rat cerebellum (unpublished observations). The present study was undertaken to investigate the effects of chronic barbital administration on cerebellar cGMP levels.

Adult female Harlan Sprague-Dawley rats were ovariectomized and feed ground lab blox admixed with increasing dosages of barbital for a 7 week time period. This feeding regimen has been shown to produce barbital dependence (Morgan, Res. Commun. in Sub. Abuse 3:177, 1982). At the beginning of the 7th week animals were given acute injections of barbital (0, 25, 50, 100, 200 mg/kg, i.v.) and activity levels were monitored for 3 min beginning 12 min after drug administration. There were no differences in activity levels between control and barbital-dependent rats. One week later animals were again challenged with acute injections of barbital (0, 25, 50, 100 mg/kg, i.v.) and sacrificed by microwave irradiation. Acute injections of barbital administered to either barbital-dependent or control rats resulted in a dose-dependent decrease of cerebellar cGMP levels. Blood barbital measurements indicated that barbital-dependent rats had average circulating barbital levels of 106±12 µg/kg prior to acute barbital injections (B-0 group). Circulating barbital in control rats receiving injections of 100 mg/kg (C-100 group) reached approximately the same level. Cerebellar cGMP levels were slightly reduced from control levels (772±53 pmol/gm tissue) in the B-0 group (560±103 pmol/gm tissue) but very significantly reduced in the C-100 group (76±8 pmol/gm tissue).

These results strongly indicate that chronic barbital administration results in tolerance as measured via the cerebellar cGMP system. Also, the tolerance appears to be closely correlated with the chronically circulating blood barbital levels since any additional barbital reduces cerebellar cGMP levels significantly. Due to the sensitivity of the cerebellar cGMP system to barbital administration it appears to be a useful system for further investigation of and insight into the mechanisms which may underlie barbiturate addiction and the withdrawal syndrome. (Supported by DA 00755 and RSDA DA00083 to WWM.)

358.21 EFFECTS OF CHRONIC EXPOSURE TO PHENOBARBITAL ON CULTURED MAMMALIAN CENTRAL NEURONS. E.E. Serrano* and B.R. Ransom. Dept. of Neurol., Stanford Univ. Sch. of Med., Stanford, CA 94305.

The anticonvulsant phenobarbital (PB) is frequently used to manage epilepsy in pregnant women and children raising concern that this drug might have effects on the developing CNS. Indeed there is evidence that this drug impairs normal neuronal development based on *in vivo* and *in vitro* experiments. We sought more detailed information about effects of this drug on the development of mammalian neurons *in vitro* in terms of cell density and individual cell morphology. Cultured mouse spinal neurons have proved a valid model for studying drug actions on CNS neurons and were utilized in these experiments (Barker and Ransom, J. Physiol. 280: 355, 1978).

Cultures were maintained in PB at concentrations of 20, 40, or 90 µg/ml from either the 2nd or 14th day after plating. Sister cultures not exposed to PB acted as controls. Cell density was evaluated in mature cultures 8 weeks old using phase microscopy. Cells were categorized as small neurons (somata < 20µ in diameter), large neurons (somata > 20µ), dorsal root ganglion cells, or included in a "summary" category including all of the above plus any other phase bright cells. Cell density was determined by counting cells over 10% of the surface area of at least three dishes in each condition. Exposure to PB produced a dose-dependent reduction in small and large neurons, and total cell density of up to 80% of control. Curiously, cultures exposed from day 2 after plating were less severely affected than cultures exposed from day 14. As a control experiment cells were exposed to barbituric acid which reduced cell density in a manner identical to an equimolar concentration of PB (172 µM). This suggests that the detrimental effects of PB may not be correlated with its anticonvulsant activity.

To determine if PB altered the morphology of cells which remained after chronic PB exposure, neurons were injected with the fluorescent dye Lucifer Yellow, photographed and analyzed with regard to somal diameter and the extent and complexity of dendritic branching. No significant differences were noted in somal diameters between control and treated neurons. The length of neuronal processes and the amount of branching were reduced in a dose-dependent manner after PB exposure. These effects developed more quickly when the cultures were exposed to PB from day 14 as opposed to a similar duration of exposure beginning from day 2.

We conclude that chronic PB exposure adversely affects mammalian central neurons maintained *in vitro*. It appears to reduce neuronal density as well as cause a simplification in the branching pattern of remaining neurons. Studies to assess the consequences of these alterations in terms of neuronal physiology, synaptic interactions, and responsiveness to various neurotransmitters are underway.

Supported by NIH grants NS 12151 and NS 00473 from the NINCDS.

358.22 ALTERATIONS IN •GLUCOSE UPTAKE IN BRAIN DURING THE WITHDRAWAL SYNDROME IN PHENOBARBITAL-DEPENDENT RATS. Cheryl A. Marietta,* Michael J. Eckardt, Henry N. Wixon,* and Forrest F. Weight. Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

In humans and experimental animals that are physically dependent on phenobarbital, a withdrawal syndrome indicative of CNS hyperexcitability can result from cessation of phenobarbital administration (Kalant,H., et al, Pharmacol Rev., 23: 135, 1971). We investigated the effect of phenobarbital withdrawal on cerebral glucose metabolism using 2-deoxy-D-[^{14}C]glucose (2-DG) as a metabolic tracer. Female Sprague-Dawley rats were rendered physically dependent by administering phenobarbital for 9 weeks by intubation with a series of stepped doses - 75 mg/kg/day for the first 2 weeks, 150 mg/kg/day for the next 3 weeks and 200 mg/kg/day for the last 4 weeks. Physical dependence was indicated by the appearance of a withdrawal syndrome characterized by hyperactivity, tremors, weight loss and an occasional spontaneous convulsion. These signs commenced approximately 16 hours after the last dose of phenobarbital was administered and peaked 24 to 30 hours after the last administration of phenobarbital. When rats appeared to show a maximal withdrawal response, local brain glucose uptake was determined by means of the 2-DG method of Sokoloff et al (J. Neurochem, 28:897, 1977). In withdrawing rats, a generalized increase in 2-DG uptake was seen in most gray areas, compared to controls. Localized increases in 2-DG were also apparent in withdrawing animals as dark columns in the frontal sensorimotor cortex and the parietal cortex, and as dark areas in the cerebellar vermis. Similar columns and areas were not seen in controls. In withdrawing animals, specific thalamic nuclei also manifested increased 2-DG compared to controls, and the lateral geniculate was banded with the dorsal portion darker than the ventral portion, whereas in controls the lateral geniculate was of uniform density. The observed alterations in metabolic activity are consistent with presumed general and localized increases in neuronal activity associated with CNS hyperexcitability in the withdrawal syndrome.

358.23 INHIBITION OF FAST-PHASE $^{45}Ca^{++}$ UPTAKE INTO SYNAPTOSOMES AND MICROSACS BY PENTOBARBITAL. E. Barr*, L.C. Daniell* and S.W. Leslie (SPON: Carlton K. Erickson) Division of Pharmacology, College of Pharmacy, Univ. of Texas at Austin, Austin, Texas 78712.

Uptake of $^{45}Ca^{++}$ by synaptosomes isolated from cerebellum, midbrain and brain stem of male Sprague-Dawley rats was measured at 3 seconds. The fast-phase, voltage-dependent (65mM KCl) component of Ca^{++} uptake by the brain regional synaptosomes was inhibited in a concentration-dependent manner by *in vitro* addition of pentobarbital (PB). PB, 0.05, 0.1, 0.2 mM, inhibited voltage-dependent $^{45}Ca^{++}$ uptake by 12.2, 17.3 and 36.0 percent for the cerebellum: 15.8, 26.8 and 45.5 percent for the midbrain and 16.0, 17.3 and 38.5 percent for brain stem. Voltage-dependent $^{45}Ca^{++}$ uptake by cerebrocortical synaptosomes was inhibited in a time-dependent manner by 0.2 mM PB. At 1,3,5,15,30 and 60 seconds uptake of $^{45}Ca^{++}$ was inhibited by 69, 49, 39, 34, 27 and 24 percent, respectively. These results support our previous findings that anesthetic concentrations of pentobarbital inhibit $^{45}Ca^{++}$ uptake by presynaptic nerve terminals. The fast-phase component of $^{45}Ca^{++}$ uptake was more sensitive to PB inhibition than the slow phase component. Uptake of $^{45}Ca^{++}$ by postsynaptic "microsacs" isolated from whole brain of male Sprague-Dawley rats was measured at 1,3,5,15 and 30 second time periods. Microsacs like synaptosomes show their fastest rates of uptake between 0 and 1 seconds when depolarized with 65 mM KCl and exhibit in the 3-5 second interval a decline of the uptake rate to 35% of the 0 to 1 second interval. Addition of 0.2 mM PB *in vitro* caused a statistically significant inhibition of voltage-dependent $^{45}Ca^{++}$ uptake at the 1, 3 and 5 second measurement times (35.2, 22.7, and 34.6 percent, respectively). Uptake at 15 and 30 seconds was not altered by pentobarbital. The inhibition of fast-phase $^{45}Ca^{++}$ uptake by synaptosomes and microsacs may be related to the anesthetic effect of PB.

ALCOHOL: BEHAVIOR

359.1 THE EFFECTS OF PRENATAL ALCOHOL EXPOSURE ON BRAIN MORPHOLOGY/HISTOLOGY AND OPEN-FIELD BEHAVIOR IN RATS. S. Poznanski* and K. King. Dept. of Psychology, Kenyon College, Gambier, OH 43022.

Prenatal exposure to alcohol in humans may result in a pattern of mental and physical deficits known as Fetal Alcohol Syndrome (FAS). A number of investigators have attempted to model this syndrome in animals--with mixed results. The purpose of this study was to further investigate behavioral and neurochemical effects stemming from *in utero* alcohol exposure.

Offspring born to mothers which consumed a liquid ethanol diet were compared to offspring born to pair-fed controls. Significant differences between these groups were found when comparing body weight, brain size, and cell density. Alcohol-treated rats were significantly lighter as pups, and as adults had significantly smaller brains (despite equal body weights). In addition, animals exposed prenatally to ethanol were found to have significantly lower densities of cerebellar Purkinje cells and hippocampal pyramidal cells.

Several indices of open-field activity were also measured in order to assess differences in activity and emotionality. Because previous research has indicated that central monoaminergic neurotransmitter systems are altered by early alcohol, animals were tested following injections of Fluoxetine (a serotonin reuptake inhibitor) and L-DOPA. Significant differences in these treatment groups were found. In some comparisons drug effects interacted with the sex of the animals; in others, significant interactions were seen between drug effects and the developmental stage of the rats. Overall, the results of this study show that prenatal exposure to alcohol results in pronounced changes in the growth of the brain which are in turn reflected in more subtle alterations in neurochemical systems and open-field behavior.

359.2 TOXIC INTERACTIONS OF ETHANOL AND PROPOXYPHENE. R.J. Reiffenstein, Manuel Mah* and T. Williams* (SPON: W.F. Dryden). Dept. of Pharmacology, Univ. of Alberta, Edmonton, AB, Canada T6G 2H7.

In 1979 a news item (Science, 203, 857) drew attention to claims that the lethal dose of propoxyphene was greatly reduced by prior consumption of ethanol (potentiation). Published animal and human data provided no support for these claims of potentiation, but only suggested that an additive effect was present. These experiments were carried out in mice to determine whether this combination of drugs was additive or mutually potentiating. For comparison purposes combinations of ethanol and pentobarbital were also examined.

ICR stain white mice of 20-30 grams were given i.p. injections of either or both drugs in a wide range of doses. From 12 to 24 mice received each dose or dose combination. Injections were given within 30 seconds of each other into two different sites. Mice were monitored for sedation, loss of righting, and death in the propoxyphene study, and anesthesia and death for pentobarbital. ED50s and LD50s for each endpoint were calculated from the dose-response curves and these values plotted on isobolograms, which is the only way of determining if drugs producing the same effect are additive or potentiating (Gessner & Cabana, JPET 174, 247, 1970). The line between the ED50s of each drug alone shows simple addition. Statistical evaluation was by exact binomial probability with the null hypothesis that the data would lie equally on both sides of this line (i.e. simply additive).

Pentobarbital was chosen as a drug which acts by a similar mechanism to alcohol, and thus most likely to be simply additive in interaction. While this proved to be true for anesthesia, the interaction was clearly less-than-additive for the lethal effect.

Propoxyphene showed a simple addition for sedation, but was infra-additive for loss of motor co-ordination (righting) ($p=0.0078$) and for lethality ($p=0.00024$). No LD50s appeared in the "potentiation" part of the isobologram (between the additive line and the origin. It is worth noting that the LD50 for propoxyphene appears to be lower than the ED50 for loss of righting. Thus the mechanism of lethal effect would appear to be different from the central depression which causes the loss of motor co-ordination. While the claims of potentiation of propoxyphene by ethanol appear to be misfounded (and the interaction is actually less than additive), the effect of any 2 doses together is greater than either dose alone, and still represents a greater hazard.

Supported by a contract from the Alberta Alcoholism and Drug Abuse Commission.

359.3 FRONTAL CORTICAL MEDIATION OF ETHANOL-INDUCED STEREOTYPY IN THE RADIAL-ARM MAZE. R.L. Hale*, D.A. Whiteside*, and L.D. Devenport (SPON: A. Revzin). Department of Psychology, University of Oklahoma, Norman, OK 73019.

Recent research has shown that one of the most powerful effects of ethanol is upon behavioral variability (BV). Ethanol (1.5-2.0 g/kg) suppresses several indices of variability of rats in the radial arm maze (Devenport & Merriman, Psychopharmacol., 1983; Devenport et al., Pharmacol. Biochem. Behav., 1983). It is of interest to know if ethanol's effect is more dependent on some brain structures than others. In the present study, the role of the rat frontal cortex in the mediation of ethyl alcohol's effect upon behavioral variability in the 8-arm radial maze was examined.

Food-deprived frontal- and parietal (control)-aspirated rats, injected with either saline or 10% (w/v) ethyl alcohol (1.5 g/kg), were run in a reward replacement regimen in the 8-arm radial maze for 16 sessions, three trials per session. This procedure allowed rats to select arms in any sequence--variable or stereotyped--without penalty. The administration of ethanol had the expected effect of suppressing sequential variability in parietal-ablated rats. However, and most importantly, ethanol lost this effect in rats with frontal damage. This was the case despite the fact that frontal lesions themselves did not diminish the variability of response patterns.

It was not simply the case that ethanol lost its potency after frontal ablations. Its usual suppressive effect on a separate measure of BV, topographic variability, was as strong in frontal- as in parietal-damaged rats. The absence of ethanol-induced stereotypy in frontal-aspirated animals suggests that the frontal cortex is critical for the mediation of this action of ethanol, but does not itself participate in the organization of sequential variability. Interestingly, this pattern of drug-brain interaction has recently been found (Solomon et al., Science, 1983) with a different drug (scopolamine) and brain structure (hippocampus). As in the present study, the behavioral effects of a drug were found to depend upon an intact brain area, but the brain region itself was not critical for the organization of the behavior.

Supported by USPHS grant AA05699-02 to LDD

359.4 SUPPRESSION OF ETHANOL WITHDRAWAL AUDIOGENIC SEIZURES BY MUSCIMOL IN THE INFERIOR COLLICULUS BUT NOT IN THE SUBSTANTIA NIGRA OR MEDIAL SEPTUM. G.D. Frye, G.R. Breese & T.J. McCown, (SPON: D.O. Hernandez). Ctr. for Alc. Stud., Biol. Sci. Res. Ctr, Univ.of N.Carolina, Sch. of Med., Chapel Hill, N.C. 27514

Activation of GABA receptors with GABA-mimetic drugs suppresses ethanol withdrawal-induced, audiogenic seizures (AGS) in physically-dependent rats (Frye et al., J. Pharmacol. Exp. Ther. in press, 1983). Recent reports indicate that the substantia nigra may be the principal site where GABA-mimetic drugs act to block a variety of types of seizures. For example, seizures evoked by electroshock, i.v. bicuculline or pentylenetetrazol injection or electrical amygdaloid kindling are blocked by muscimol (10-30ng) injected bilaterally into the substantia nigra (Iadarola and Gale, Neurosci. Abst. 7:591, 1981; McNamara et al.,Neurosci. Abst. 8:86, 1982). In the present study muscimol (30-100ng) microinjected bilaterally (0.1ul/min over 5 min) into the substantia nigra 15 or 30 min before testing failed to suppress AGS in physically-dependent rats withdrawn from ethanol for 7-8hr. Bilateral microinjection of muscimol (10-30ng) into the inferior colliculus completely suppressed AGS wild running, clonus and tonus, for a period of 15-60 min. The incidence of AGS was still reduced 3hr but not 5hr after muscimol (30ng). Microinjection of muscimol (30-100ng) into the medial septum, an area in which GABA antagonists can reduce barbiturate-induced depression (Brunello and Cheney, J. Pharmacol. Exp. Ther. 219: 489, 1981) did not block AGS. GABA (10ug), THIP (300 ng) and chlordiazepoxide (10-30 ug) microinjections in the inferior colliculus also blocked AGS, but 1,3-butanediol was inactive. AGS-like wild running, clonus and tonus were evoked in ethanol-naive rats during bilateral microinfusion (0.1ul/min for 6 min) of bicuculline methiodide (BICMETH; 6.0ng but not 1.8ng), while BICMETH (600ng but not 180ng) in the substantia nigra caused primarily myoclonus and clonic-tonic seizures with little wild running. In the medial septum, BICMETH (1.8ug) infused over 6 min. did not cause any seizure-like activity. These data suggest that the inferior colliculus may play an important role in the action of GABA-mimetics to suppress ethanol withdrawal AGS which may result from reduced GABAergic activity in this nucleus during withdrawal. Supported in part by PHS AA05713; AA02334, and NC. ALC. RES. AUTH. 8019, 8207.

359.5 BICUCULLINE-INDUCED SEIZURES IN MICE WHICH DIFFER IN SENSITIVITY TO ETHANOL. T.J. Phillips* and B.C. Dudek. Dept. Psychology and The Neurobiology Research Center, SUNY-Albany, Albany, NY 12222.

Bicuculline-induced seizures were assessed in Long-Sleep (LS) and Short-Sleep (SS) mice, two lines selectively bred for response to sedative-hypnotic effects of ethanol (ETOH)(McClearn & Kakihana, Behav. Genet. 3:409, 1973), and their F1 hybrids. When administered ETOH, SS mice become less hypothermic, are less depressed by hypnotic doses and more activated by subhypnotic doses, but metabolize ETOH at a rate similar to the LS mice. Previous studies of convulsive behavior in these mice have demonstrated more severe ETOH-withdrawal reactions in SS than in LS mice; latency to onset of flurothyl-induced seizures has also been reported to be shorter in SS mice.

SS mice were significantly more susceptible to bicuculline -induced seizures (4.0mg/kg i.p.) than LS mice; SS mice had shorter latencies for both the myoclonic and clonic seizure components. The pattern of F1 inheritance varied with type of seizure. F1 mice had myoclonic latencies which were intermediate to the two parental lines, but the clonic latencies were similar to the less susceptible LS mice. In earlier work, the pattern of F1 inheritance was intermediate in tests of behavioral response to ETOH.

Seizure latencies in response to bicuculline were also measured in several inbred mouse strains. C57BL/6Abg mice were generally less sensitive to the convulsive effects of bicuculline than were DBA/2Abg or BALB/cAbg mice and F1 hybrids displayed dominance toward the C57BL/6Abg phenotype. This outcome parallels the data from the LS and SS mice since C57BL/6Abg mice have been characterized as having greater neurosensitivity to ETOH. A correlation is therefore indicated between the depressant response to acute ethanol challenge and resistance to seizures.

Since bicuculline is a GABA antagonist and this transmitter has been implicated in the depressant effects of ETOH, an hypothesis of LS-SS differences in GABA function is plausible. Such an hypothesis would suggest that the GABA-based hyperexcitability of the SS central nervous system is reflected both in their shorter seizure latencies and their relative insensitivity to the depressant effects of ETOH. Further work on the interactive effects of ETOH and GABA antagonists is in progress.

Supported by the Research Foundation of the State University of New York

359.6 DRUG DISCRIMINATION ANALYSIS OF THE DUAL BEHAVIORAL EFFECTS OF ETHANOL. T.S. Shippenberg*, E. Knappenberger* and H.L. Altshuler. Neuropsychopharmacology Section, TRIMS and Department of Pharmacology, Baylor College of Medicine, Houston, Tx. 77030.

In rats, ethanol (ETOH) administration produces transient behavioral excitation that is followed by sedation. The initial excitatory effect occurs shortly after low doses (0.2 - 1.0 gm/kg and is often quite distinct from the later onset, high dose sedative effect. This study utilized a drug discrimination (DD) procedure to characterize the biphasic effects of ETOH in greater detail. Forty male, Sprague - Dawley rats (100 - 120 gm) were trained to discriminate ETOH (1.0 gm/kg, 10% w/v, IP) from saline (SAL: 1.0 ml/100 gm, IP) on a fixed - ratio 10 (FR-10) reinforcement schedule in a 2 lever operant paradigm. Twenty rats were trained to perform the discrimination during ETOH's excitatory phase of action (6 min post-dose) and 20 rats were trained to perform the discrimination during ETOH's sedative phase (30 min post-dose). After rats had achieved the performance criterion (90% drug appropriate responses), dose - response curves (ETOH: 0.6 - 1.2 gm/kg) were generated. DD was then tested at various times post dose (6 - 60 min) using a range of ETOH doses (0.6 - 1.2 gm/kg). Blood and brain ETOH concentrations were determined with gas chromatographic techniques at each dose and time post dose. The role of opiate pathways in producing the ETOH intereoceptive cue (IC) was examined by testing DD after administration of opiate agonists or after opiate receptor blockade. DD was tested after IP administration of naloxone (NLX: 2.5 - 10 mg/kg); NLX (2.5 - 10 mg/kg) and ETOH; or morphine (2.0 - 7.5 mg/kg). Results from these studies demonstrate that the IC associated with ETOH's excitatory effect did not generalize to the IC associated with ETOH's sedative effect in rats trained to discriminate ETOH's sedative effect. Similarly, ETOH's sedative effect did not generalize to ETOH's excitatory effect in rats trained to discriminate ETOH's excitatory effect as evidenced by the SAL appropriate lever responses produced. Differences in blood ETOH concentrations did not account for the different ICs produced. Additionally, there was no generalization between morphine and ETOH, and NLX did not antagonize the IC associated with ETOH's sedative effect. These data demonstrate that the IC associated with ETOH's excitatory effect differs from the IC associated with its sedative effect. In addition, these results demonstrate that blockade of opiate receptors during ETOH's sedative phase does not antagonize ETOH's sedative effect.

359.7 INCREASE IN VOLUNTARY ALCOHOL DRINKING AFTER ELEVATION OF ENDOGENOUS ALDEHYDES BY CYANAMIDE. E.C. Critcher*, J.R. Hepler* & R.D. Myers, Center for Alcohol Studies and Departments of Psychiatry and Pharmacology, University of North Carolina School of Medicine, Chapel Hill, N.C. 27514.

The rationale of inhibiting aldehyde dehydrogenase (ALDH) by drug treatment is an approach which has been used clinically to prevent alcohol drinking, and similarly to reduce voluntary alcohol consumption in the experimental animal. We now report that chronic treatment with cyanamide, an ALDH inhibitor, can permanently increase alcohol intake when the availability of alcohol is dissociated from injections of this drug. The baseline intake of alcohol was determined over 7 days by offering water and a single concentration of alcohol, ranging from 5% to 18%. Then, alcohol was removed for the four days of cyanamide treatment and for two additional days. Either cyanamide in a dose of 0.03, 0.1, 0.3, 0.5 or 1.0 mg or an artificial CSF was infused intracerebroventricularly (ICV), through previously implanted cannulae, three times on each of four consecutive days. In parallel experiments, either cyanamide in a dose of 10, 20 or 30 mg/kg or saline control vehicle was injected subcutaneously (SC) according to the same paradigm. On the third day after either treatment regimen, alcohol was again made available. The intake of alcohol was enhanced substantially in those animals treated either SC with 10 mg/kg cyanamide or ICV with 0.1, 0.3 or 0.5 mg of this drug. The two remaining doses of cyanamide given peripherally were either lethal or caused a permanent suppression of alcohol drinking. On the other hand, the 0.03 and 1.0 mg doses of the ALDH inhibitor given ICV failed to alter alcohol drinking. Changes in alcohol intake persisted for 6 weeks or longer following cyanamide administration. These findings suggest that the elevation of endogenous aldehyde in the brain and/or in the periphery produces a sustained increase in voluntary alcohol drinking. Since this is an effect similar to that produced by centrally injected tetrahydroisoquinolines, these results correspond to the theory that an alteration in alcohol drinking may be due to the biosynthesis of a condensation product, the formation of which would be facilitated by an enhanced aldehyde level. (Supported in part by NIAAA Grant R01 AA 04200 03, by ARA Grant #8102 and by an ARA Fellowship Grant #8202.)

359.8 CONDITIONED PLACE PREFERENCE AND ETHANOL. Karen E. Asin, David Wirtshafter and Boris Tabakoff, Dept. Physiology & Biophysics and Dept. Psychology, U. IL., Chicago, IL. 60680.

Although ethanol is a major drug of abuse in man, its reinforcing properties have generally been difficult to demonstrate in lower animals. Two bottle preference tests may be confounded by differences in solution palatability, and reports of ethanol self-administration by nondependent rats have largely been equivocal.

A relatively rate-free measure of reinforcement may be obtained through the use of the place preference paradigm (PPP), where the reinforcing properties of a drug are classicially conditioned to place cues. Some studies have attempted to demonstrate ethanol's reinforcement properties using the PPP, again with equivocal results. Theoretically, it should be possible to demonstrate an ethanol-induced place preference, since the reinforement properties of other drugs of abuse have been demonstrated using the PPP. We therefore sought to more clearly define the parameters through which an ethanol-induced place preference might be obtained.

Adult, naive rats were tested for original side preference in a shuttle box with easily discriminable compartments. Rats were then confined to one side and were administered either 0, .15, .30, .60, .80 or 1.00 g/kg (i/p.) ethanol (12%v/v) in the non-preferred side or distilled water in the preferred side on alternate days for 8 days. On the following day, rats were again allowed to traverse the apparatus and time spent in each compartment was recorded. The change in time spent in the originally non-preferred side was used as an index of the reinforcement efficacy of ethanol. Statistical analysis indicated that none of the doses of ethanol produced a reliable change in side preference compared to controls, although occaisional rats (12%) in the higher doseage groups showed large changes in preference.

In a further attempt to demonstrate ethanol's reinforcing properties, rats were prepared with indwelling jugular cannula and were trained on the PPP using 1.0, 2.0 or 8.0 mg/kg i.v. ethanol. Preliminary results suggest that animals treated with the lower doseages show changes in place preference compared to controls. These results suggest that the reinforcing properties of ethanol may be demonstrated using a PPP is small doseages, similar to those which promote self-administration by rats, are used.

Supported by NIAAA.

359.9 THE EFFECTS OF PROSTAGLANDIN SYNTHESIS INHIBITION ON PHYSIOLOGICAL AND BEHAVIORAL MEASURES OF THE ALCOHOL WITHDRAWAL SYNDROME IN DBA/2J MICE. D. M. Gilliam and A. C. Collins*. Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309.

Inhibition of prostaglandin synthesis has been shown to antagonize ethanol-induced narcosis, hypothermia, and behavioral activation, while prostaglandin administration has been found to reduce the severity of alcohol withdrawal seizures in mice. In order to investigate how inhibition of prostaglandin synthesis may affect the alcohol withdrawal syndrome, male DBA/2J mice were exposed for 9 days to either a control diet or a diet containing 26.5 mg/ml ethanol and were tested for pulmonary ventilation rate (respiration rate), acoustic startle response, and body temperature at 1.5, 4.0, 8.5, 11.0, 26.0, and 50.0 hr after the end of the treatment period. In addition, body weight was assessed at 0.0, 26.0, and 50.0 hr and heart rate was measured at 6.0 hr after termination of treatment. Control- and ethanol-fed mice were injected at 0.5 hr before the first post-treatment test with either a control solution or a solution containing 12.5 mg indomethacin/kg body weight.

Control- or indomethacin-injected mice that had received the control diet during the treatment period did not differ on any measure at any time. Mice fed the ethanol diet exhibited an overall depressed respiration rate, startle response, body temperature, and heart rate in comparison with control-fed mice, regardless of the injection condition. However, ethanol-fed, indomethacin-injected mice showed baseline body temperature and a significant weight gain compared to ethanol-fed, control-injected mice at 26.0 hr. Indomethacin may act as an anti-inflammatory agent as well as an antipyretic agent, with its assuasive actions permitting food consumption during the early stages of withdrawal and its antifebrile actions, coupled with an adequate diet, allowing a return to normal body temperature regulation within 24 hr after chronic ethanol treatment.

359.10 LONG-TERM TOLERANCE TO ETHANOL'S EFFECTS ON OPERANT PERFORMANCE IN THE RAT. F.A. Holloway, D.C. Bird*, and J.A. Holloway. Dept. of Psychiatry and Behavioral Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190 USA

We previously reported that chronic exposure to ethanol resulfeted in the development of persistent tolerance to ethanol's effects on schedule-controlled behavior (Alcoholism: clin. exp. res. 1981, 5(1), 143). In that study, male albino rats given daily (4-6 weeks) i.p. injections of ethanol (1.5-3.0 g/kg) just prior to food-reinforced operant sessions showed a shift to the right in their ethanol dose-effect curve, a shift which was still evident 6 months post-withdrawal. Such a long-term effect suggested the possibility of a conditioned tolerance effect in which the injection procedure might serve as a conditioned stimulus.

The present study sought to minimize classical conditioning effects by chronically exposing rats (n=4) to ethanol with a standard liquid-diet procedure (BioServ; 40-50% ethanol as calories) for a period of three weeks. Controls rats (n=4) received an isocaloric (maltose-dextrin added) amount of liquid diet. Rats were trained to criterion performance on an operant task where a food pellet was delivered after 30 lever presses (FR30). Dose-effect curves for ethanol were obtained prior to the period on the liquid diet, during the first 2 weeks after withdrawal from ethanol (both groups still on the diet), and 6 months after withdrawal. During the latter 6 months, operant sessions (always 15 min) continued with an ethanol challenge (1.5 g/kg) once a month.

At all test times, both groups displayed dose-related decreases in responding. The control group displayed almost a complete overlap in the 3 dose-effect curves. The ethanol-diet groups showed a shift in the right in their post-diet dose-effect curve with only partial recovery 6 months after withdrawal.

We then sought to determine whether our ethanol rats would display other persistent behavioral effects. Thus, after the final ethanol dose-effect curve was obtained, several additional biobehavioral assays were given all rats. The two groups showed no difference on our operant task in dose-related sensitivity to caffeine, no difference in ethanol- or stress-induced analgesia, and no difference in their peak blood ethanol level or elimination rate. However, ethanol rats showed impairment in avoidance conditioning, higher preference for ethanol, and decreased sensitivity to ethanol on a dissimilar "exploratory" task.

These data suggest that the chronic ethanol procedure produced very long-term changes not only in the rats' sensitivity to ethanol, but also in non-drug related learning/performance capabilities. Further, the operant data do not readily lend themselves to an interpretation of either metabolic tolerance or an environmentally dependent tolerance. The results do suggest some residual alteration in CNS functioning.

359.11 ENHANCED RESPONSIVENESS TO MORPHINE IN ADULT RATS FOLLOWING FETAL ETHANOL EXPOSURE. L.R. Nelson, J.W. Lewis, J.C. Liebeskind, N. Kokka*, D. Randolph*, B.J. Branch*, and A.N. Taylor. Departments of Psychology and Anatomy, and Lab. of Neuroendocrinology, Brain Research Institute, UCLA, and Brentwood VA Medical Center, Los Angeles, CA 90024.

We have shown that fetal ethanol exposure (FEE) results in enhanced responsiveness to stress in adulthood. Specifically, FEE rats release more corticosterone (CS) in response to some stressors and display greater opioid-mediated, stress-induced analgesia. In the present study, we assessed these same responses (CS elevation, and analgesia) to an exogenous opiate, morphine. Additionally, we investigated the thermic response to morphine, since we have shown that FEE rats display enhanced thermic responses to diazepam, phenobarbital, and ethanol.

Subjects were female offspring of Sprague-Dawley dams fed either a 5% w/v ethanol-containing casein-supplemented liquid diet (BioServ) ad lib (FEE), pairfed an isocaloric liquid diet without ethanol (PF), or given lab chow and water ad lib (N), from gestation day 8 to birth.

Rats approx. 180 days of age were tested for morphine analgesia using the tail-flick procedure. Baseline tail-flick latencies were similar in all three groups. Following a low dose of morphine (1.25 or 2.5 mg/kg s.c.), testing was repeated at 30, 60, 90, and 120 min. FEE rats showed greater morphine analgesia (2.5 mg/kg dose) than N or PF rats at all time points ($p < .01$). No group differences were found with the 1.25 mg/kg dose.

Rats approx. 120 days of age were tested for body temperature changes following a moderate dose of morphine (10 mg/kg i.p.). Rectal temperatures were recorded prior to morphine injection and at 30 min intervals for 3 hrs thereafter. This dose of morphine did not affect body temperature in N and PF rats during the first 2 hrs post-injection but caused a slight hyperthermia during the 3rd hr. FEE rats, however, demonstrated hypothermia and differed from N or PF rats at 90, 120 and 150 min post-injection ($p < .01$).

Rats approx. 210 days of age were tested for CS elevation in response to a high dose of morphine (20 mg/kg i.p.). At 1 hr post-morphine, rats were sacrificed and trunk blood was collected for CS determination by a fluorometric method. FEE rats had significantly higher CS levels than N or PF rats following morphine ($p < .05$).

These results suggest that prenatal exposure to ethanol leads to widespread changes in endogenous opioid systems since a variety of responses to morphine are augmented in FEE rats. (Supported by VA Medical Research Service and NIH grant NS07628, L.R.N. was supported by MHTP grant MH 15345.)

359.12 SIMULTANEOUS TOLERANCE AND INTOLERANCE TO TWO EFFECTS OF ETHANOL IN RATS SUGGESTS A LEARNING ROLE. D.L. HJERESEN, D.R. REED* AND S.C.WOODS. Psychology Dept., University of Washington, Seattle, WA 98195

In recent years a variety of learning mechanisms have been implicated in the development of drug tolerance. We have previously demonstrated that when rats are deprived of hypothermic cues by microwave hyperthermia (MHT) no tolerance to ethanol hypothermia develops. Futher, such rats cease to be tolerant to ethanol hypothermia when MHT is introduced (Hjeresen, D.L.,Reed, D.R.,Woods,S.C., Alcoholism:Clin. Exp. Res.,70:111 1983). In the present experiment we demonstrate that rats which do not become tolerant to ethanol hypothermia due to MHT do become tolerant to the ataxic effects of ethanol.

Four groups of male Long-Evans rats were tested (Mean Body Mass=198+10 g). Group 1:Saline injection/Sham MHT; Group 2: Saline injection/MHT; Group 3:Ethanol (2.5 g/kg, 15% v/v)/Sham MHT; Group 4: Ethanol/MHT For 7 days rats were handled daily and pretrained on a treadmill task. In a 38x32x21 cm. Plexiglas chamber, rats were required to walk on a moving (4cm/sec), 6.4 cm. wide stainless steel belt in order to avoid an electrified grid. Each session consisted of 3-60 second trials with a 30 sec. ITI. Daily procedures for Days 8 through 18 were as follows: Rectal temperature, ethanol or equivolume saline injection, 30 mins of MHT or sham MHT, a second rectal temerature, and 3 treadmill trials. On Day 19 all rats were injected with ethanol after an initial rectal temperature and sham heated for 30 minutes. A second temperature was taken and the rats were tested on the treadmill.

	DAY 8-18 $\bar{X}$ Temp change ^{o}C	DAY 18 $\bar{X}$ sec. off TM	DAY 19 $\bar{X}$ Temp change ^{o}C	DAY 19 $\bar{X}$ sec. off TM
GRP1	0.8±0.1	2.8±1.8	-0.8±0.1	25.4±5.8
GRP2	2.3±0.2	1.1±0.4	-1.4±0.2	20.5±4.3
GRP3	-0.6±0.1	1.0±0.4	0.1±0.1	0.6±0.2
GRP4	2.1±0.1	0.6±0.1	-0.9±0.1	1.4±0.4

Rats in Group 4 which were injected with ethanol but not made hypothermic, were as intolerant to ethanol hypothermia as saline controls on Day 19. These same rats were as tolerant to the ataxic effects of ethanol as rats in Group 3. This suggests that tolerance to an effect of ethanol is dependant on physiological cues specific to that effect.

359.13 BEHAVIORAL STUDIES OF ADULT RATS TREATED WITH ETHANOL ON POSTNATAL DAYS 1-8. A. J. Ritchie* and T. B. Sonderegger, Dept. of Psychol., U. Nebraska-Lincoln, Lincoln, NE 68588-0308.

Recent studies have reflected a growing concern within the scientific and public communities as to the long-term effects of ethanol on the developing nervous system. While most of these studies have examined the in-utero effects of ethanol administration, the study reported here utilized an intragastric intubation method to administer known quantities of ethanol to rat pups on postnatal days 1-8. Ethanol was in a 30% Sustagen vehicle (Mead Johnson) to counteract underfeeding effects (Sonderegger et al., Neurobehav. Tox. and Teratol. 4:483, 1982).

Pups from eight litters of Charles Rivers CD albino rats, adjusted to 10 pups each, were randomly assigned to treatment groups: ethanol (E), Sustagen (S) or pair-underfed (P). Ethanol was given in tapered doses to E pups, twice daily, increasing to a maximum of 4 g/kg on days 4 and 5. S pups received isocaloric (sucrose) Sustagen in comparable volumes. Since E pups ceased to gain weight on day 4, handled littermates (P) were removed from their litters and placed with nonlactating females until body weights matched those of their E littermates. Animals were weaned on day 21 and housed in same-sex pairs. Although E and P animals had low body weights on postnatal day 8, group mean weights did not differ through day 150.

Adult animals were subjected to two learning tasks (passive avoidance and the Lashley III maze) and a photocell open field activity measure. The passive avoidance task showed no significant differences between groups nor did 20-hour photometer activity measures. A significant number of ethanol-treated animals failed to master the Lashley III maze, a finding consistent with impairments seen with early postnatal treatment of rats with other drugs such as opiates. Other findings, such as the animals' reactions to challenge doses of ethanol, will also be discussed.

Support: U. Nebraska-Lincoln Research Council and NIH Biomedical support RR07055.

359.14 MEASURES OF CNS.EXCITABILITY IN MOUSE LINES SELECTIVELY BRED TO BE ETHANOL WITHDRAWAL-SEIZURE-PRONE AND -RESISTANT. J. C. Crabbe, Jr., J. D. McSwigan*, A. Kosobud*, B. R. Tam*, and E. R. Young*. VA Med. Ctr. and Ore. Health Sci. Univ., Portland, OR 97201.

We are developing lines of mice prone (WSP) and resistant (WSR) to ethanol withdrawal seizures by within-family selective breeding for severity of handling induced convulsions (HIC) after inducing ethanol physical dependence by forced inhalation of ethanol vapor. After 5 generations of selection, WSP mice and WSR mice differ several fold in HIC severity after a standard ethanol inhalation exposure. We report experiments designed to test the hypothesis that these lines of mice differ in general arousal and/or CNS excitability not specific to ethanol withdrawal hyperexcitability.

Naive mice from second and later litters from Generations S_5 to S_7 were tested for development of tonic hindlimb extensor seizures after ip administration of pentylenetetrazole in doses of 70-105 mg/kg. The $CD_{50} \pm SE$ was found to be 91 ± 3.2 mg/kg and 94 ± 2.7 mg/kg for WSR and WSP mice, respectively, a non-significant difference. Studies with the convulsants strychnine sulphate and picrotoxin also yielded no difference in seizure susceptibility between the selected lines. In two experiments, mice that had been made ethanol dependent and withdrawn were given metrazol 4 or 9 weeks later and a trend toward enhanced sensitivity in the WSP line was seen. However, no difference between the lines in sensitivity to picrotoxin seizures was seen 9 weeks after ethanol withdrawal.

In previous studies, we have shown that the HIC, which serves as the basis for the genetic selection, is known to occur in naive mice and that it is slightly elevated by three daily injections of 1 mmol/kg pyrazole, the alcohol dehydrogenase inhibitor used in the inhalation paradigm. We tested naive WSP, WSR, and WSC (control) mice for HIC without pyrazole and found very small differences in HIC severity. Pyrazole enhanced HIC slightly in one WSP line. To test one measure of sensitivity to ethanol's CNS depressant effects, we injected mice ip with 3.5 g/kg EtOH and measured the duration of loss of righting reflex (LORR). WSP and WSR mice did not differ in 2 studies.

These results suggest that the WSP and WSR lines do not differ markedly in CNS excitability (convulsant thresholds or sensitivity to LORR induced by ethanol) unless it is enhanced by ethanol withdrawal. A small difference in HIC severity between the lines (with or without pyrazole treatment) appears to be emerging as a correlated response to selection. Its magnitude is not enough to account for the large differences between the lines in ethanol withdrawal seizure severity.

Supported by the VA and Grant DA 02799 from NIDA.

359.15 SUPPRESSION OF ETHANOL WITHDRAWAL SEIZURES THROUGH MANIPULATION OF NIGRAL GABA ACTIVITY. L.P. Gonzalez and M.K. Hettinger. Alcohol and Drug Abuse Research and Training Program and Department of Physiology and Biophysics, University of Illinois at Chicago, Health Sciences Center, Chicago, IL 60612.

Use of the benzodiazepines in the effective treatment of the ethanol withdrawal syndrome has led to the suggestion that GABAergic neurons may be involved in the modification of withdrawal symptomatology. In addition, GABAergic neurons of the substantia nigra have recently been implicated in the modulation of central seizure activity and convulsions, important components of the ethanol withdrawal syndrome. This suggests the possibility that these GABAergic neurons may be regulators of ethanol withdrawal seizures and related symptomatology. To evaluate the role of nigral GABA-receptive neurons in the ethanol withdrawal syndrome, we examined the effects of local injections of the GABA agonist, muscimol, on the occurrence of withdrawal seizures.

In this study, male, Sprague-Dawley rats received chronic bilateral implants of 25 gauge guide cannulae placed just above the substantia nigra zona reticulata (SNR). One week after surgery, the animals received chronic ethanol exposure in ethanol-vapor inhalation chambers. Following ten days of chronic exposure, the animals were removed from the chambers and were observed for evidence of withdrawal hyperexcitability. Eight hours after removal from the ethanol inhalation chamber, animals received bilateral injections (0.5 µl, x 2) into SNR of either saline or muscimol (1.0-30.0 ng, x 2). Twenty minutes after injection, and at two-hour intervals thereafter, animals were tested for susceptibility to audiogenic seizures induced by exposure to a 30 second auditory stimulus.

Muscimol was found to significantly inhibit audiogenic seizures, particularly at the highest doses tested. This protection against seizures was evident 20 minutes after injection, and lasted two to four hours post-injection. After this initial suppression of induced seizures, muscimol did not alter total mortality resulting from spontaneous seizures during a period of 24 hours post-withdrawal. These results support our initial suggestion that nigral GABA-receptive neurons may be involved in the regulation of ethanol withdrawal seizure activity. We are currently investigating the relationship between this inhibition of behavioral convulsions and central electrophysiological seizure activity.

[Supported in part by NIAAA, Grant No. PHS AA 7374-03.]

359.16 AN EFFECT OF ETHANOL ON BEHAVIOR MAINTAINED BY ELECTRICAL BRAIN STIMULATION. H.E. Criswell and C.H. Newby*. Dept. of Psychol., E. Tenn. St. Univ., Johnson City, TN 37614.

Attempts to demonstrate an effect of alcohol on operant behaviors maintained by the use of electrical brain stimulation (EBS) as a reinforcer have generally produced negative results. It is striking to observe an ataxic rat pressing a lever which initiates EBS at the same rate as he pressed it in the drug-free state. In our laboratory, we have oberved mice, which had not recovered their righting reflex following ether anesthesia, emit nose poke responses reinforced by EBS. Recent studies employing rate free measures of reinforcement efficacy have suggested that response rate is an insensitive measure of reinforcement strength (Kornetski and Esposito, Fed. Proc. 38, 1979). Accordingly, we have examined the effect of ethanol on EBS using a rate-free measure.

When an animal is placed in a shuttle box where moving to one side initiates EBS and moving to the other side terminates it, the animal can independently control the duration of the EBS (ON TIME) and time between stimuli (OFF TIME). Rate of shuttling is free to vary allowing independence of the other two parameters. We now show that, at doses of ethanol high enough to produce ataxia, mice maintain a constant ON TIME and decreased OFF TIME. This effect appears to be independent of changes in activity level.

To determine the effect of ethanol on activity level, 18 adult, female ICR mice were pretreated with either 0, 0.75, 1.5 or 3 mg/kg of ethanol i.p. and placed into shuttle boxes. Median shuttle times were recorded for 15 minutes. Shuttle times were decreased significantly following 0.75 and 1.5 mg/kg but returned to the non-drugged level at 3 mg/kg. Animals at the highest dose showed severe ataxia.

To examine the effect of ethanol on EBS, 8 mice with 36 gauge bipolar electrodes implanted into the lateral hypothalamus (AP=-0.5; L=0.5; D=4.7) were trained over a 3 day period to control ON and OFF times. The fourth day, mice were tested for 3 consecutive 15 minute periods. Ethanol (3 mg/kg) was administered at the end of the first 15 minute period. ON and OFF times were compared for the three periods using analysis of variance. ON times were not affected by the ethanol ($p>0.05$), but OFF times, which decreased significantly ($p<0.01$) following the drug treatment, returned partially to baseline during the following 15 minute period. The selective effect on OFF times, at a dosage which did not previously alter activity level, suggests a change in reinforcement efficacy.

359.17 ETHANOL-INDUCED STEREOTYPY IN THE RADIAL-ARM MAZE IS NOT MEDIATED BY THE HIPPOCAMPUS. D. A. Whiteside*, R. L. Hale*, L. D. Devenport (SPON. J. A. Devenport), Dept. of Psychology, University of Oklahoma, Norman, OK 73019.

The behavioral actions of alcohol are similar to those produced by hippocampal lesions. This being the case, the hippocampus is often suspected of mediating ethanol's behavioral effects. The present study assessed this question directly by determining the extent to which a robust effect of ethanol is eliminated by hippocampal damage.

Rats with hippocampal lesions or neocortical ablations were injected with 10% (w/v) ethanol at a dose of 1.5 mg/kg, or isotonic saline. Animals were tested 13 min after injection in an eight-arm radial maze where a reward replacement regimen was used. The radial maze was chosen as it allows for a measure of behavioral variability that has previously been shown to be sensitive to ethanol (Devenport et al., 1983 Psychopharmacol.) and hippocampal or fornix damage (Olton & Wertz, 1978 Physiol. & Behav.). Following 16 sessions (three trials per session) of behavioral testing and data collection, the rats were sacrificed for histological assessment. Sequential variability--the flexibility vs. stereotypy of routes taken from arm to arm--was expressed by the information statistic, H_2. Statistical analysis found a significant depression of sequential variability as a consequence of both ethanol administration and hippocampal damage. However, ethanol's effect was as dramatic for hippocampal-lesioned animals as it was for neocortical-ablated controls.

Despite the closely similar behavioral effects of hippocampal damage and ethanol administration, our research indicates that ethanol acts independently of the hippocampus in promoting stereotypy in the radial-arm maze. This is in keeping with an earlier report (Devenport et al., 1981 Behav. Neur. Biol.) that found independence between the hippocampus and alcohol for exploratory behavior. Additional measures of exploration in the present study confirmed this finding and lent further support to the notion of independence between hippocampus and ethanol.

Supported by USPHS grant AA05699-02 to LDD.

359.18 RETENTION OF LEARNED ALCOHOL AVERSIONS FOLLOWING GUSTATORY NEOCORTEX LESIONS. S. W. Kiefer and G. J. Lawrence*. Department of Psychology, Kansas State University, Manhattan, KS 66506

Ablation of gustatory neocortex (GN) in rats disrupts retention of preoperatively-learned taste aversions (Braun, Kiefer, & Ouellet, Exp. Neurol., 1981, 72, 711-716). In the present experiment, the effects of GN lesions on the retention of learned alcohol aversions was determined.

Twenty one male rats were placed on a schedule of restricted fluid access and trained to avoid a 6% alcohol solution by pairing the alcohol with LiCl intubation (3% body weight of .15 M LiCl). Following training, the rats were divided into two matched groups; one group (n=11) was given lesions of the GN using an electrolytic technique (see Lasiter, Physiol. Psych., 1982, 10, 377-383). The control group (n=10) received no surgical manipulation. One month of postoperative recovery was followed by a return to the restricted fluid access schedule. All rats were presented with the alcohol solution in a series of five extinction trials. Behavioral verification of the lesions then was tested: Rats were presented with .12 M LiCl in the drinking tubes and given the same solution two days later to determine if an aversion was formed.

On the whole, the lesions were relatively small but centered on the gustatory area. One lesion was deemed incomplete and the data from this rat were eliminated. All rats acquired strong alcohol aversions in a single trial. Postoperatively, GN lesions did not disrupt retention of the learned alcohol aversion. Like normal rats, rats lacking GN consumed little alcohol on the first retention test. There was a slight tendency for the operated rats to consume more alcohol than controls on the last two extinction tests. There was a marginal difference between the rats with GN lesions and controls on the LiCl test. The operated rats consumed a mean of 5.7 ml (+ 1.0) while the controls only consumed a mean of 3.7 ml (+ .5) ($t(18)=1.75$, $p < .05$).

The results indicate that GN lesions do not disrupt retention of learned alcohol aversions, a finding in contrast to that reported for other basic tastes (e.g., sucrose). For rats, alcohol solutions may involve more than simply gustatory qualities; odor probably is a strong cue and it may be this aspect of the alcohol that survives the lesion. The data from the present study may reflect the small lesions that were employed, a result consistent with the small difference between GN rats and control rats on the LiCl consumption test. Larger lesions may produce deficits in retention of alcohol aversions, a possibility presently being examined.

Supported by Kansas State University.

359.19 NEONATAL CEREBELLECTOMY ALTERS ETHANOL-INDUCED SLEEP TIME OF SHORT SLEEP, BUT NOT LONG SLEEP MICE. M.R. Palmer, L. Olson*, T.V. Dunwiddie, B.J. Hoffer* and A. Seiger*. (SPON: D.G. Whitlock). Dept. of Histology, Karolinska Institute, Stockholm, Sweden; Alcohol Research Center, Dept. of Pharmacology, Univ. of Colorado Health Sciences Center and Denver Veterans Administration Medical Center, Denver, Colorado, 80262, USA.

The effects of neonatal cerebellectomy on ethanol-induced sleep times in long sleep (LS) and short sleep (SS) mice were investigated. Cerebellectomy did not alter the ethanol sensitivity of the righting reflex in LS animals. In contrast, SS mice became more sensitive to alcohol after cerebellectomy. Even so, large differences were still observed between the alcohol-induced sleep times of cerebellectomized LS and SS mice. The data in this study indicate that, while the cerebellum must have a prominent influence on alcohol sleep time in SS animals, this brain structure is not solely responsible for the observed differences in righting reflex sensitivity to ethanol in these two mouse lines. Based on these observations, we postulate the existence of noncerebellar neurons with differential sensitivities to the depressant effects of ethanol in LS and SS mice. (Supported by USPHS grant AA-03527, by V.A. Research Service Award #394463116-01, by Swedish Research Council Grants 14P-5867, 14X-03185, 14P-0665, 25X-06326, and 14F-6314, Magnus Stiftelse, Karolinska Institute Fonder, and by the Expressens Prenatal Research Foundation.)

359.20 PENTYLENETETRAZOL INDUCED SEIZURES IN RAT PUPS PRENATALLY EXPOSED TO ALCOHOL. Jaw-Sy Chen,* Grace Shum* and Gordon A. Barr* (SPON: E.B. Gardner). Department of Psychology, Hunter College, CUNY and Department of Psychiatry, Albert Einstein College of Medicine, New York, NY.

There is ample evidence to indicate that withdrawal from alcohol can increase susceptibility for seizures. This lowered treashold may be transient, and there are data to suggest a long term protection against pentylenetetrazol (PTZ) induced seizures. The studies examining the offspring of mothers given alcohol while pregnant are less clear. Two studies suggested decreased threshold to audiogenic seizures but another reported less mortality after high doses of PTZ. The goal of the present experiment was to examine the seizure threshold to PTZ in very young pups born to alcohol treated dams.

The study was conducted on 2 and 9 day old pups whose mothers had consumed liquid diets containing 35% or 0% ethanol derived calories or standard lab chow during pregnancy. The 0% group was pair fed to minimize effects of decreased food intake. Pups were tested at 2 and 9 days of age with a challenge of 20,40,60, or 80 mg/kg PTZ injected intraperitoneally in a volume of 1 ml/100 g, and rated for seizure activity on a 6 point scale ranging from no activity to full clonic-tonic seizures with loss of righting. The observations were made every 5 minutes for 1 minute for an hour. Pups were tested but once.

Both 2 and 9 day old rat pups in all treatment groups showed a clear dose related increase in seizures. The older pups showed more seizures than did the 2 day olds. The offspring of the alcohol treated dams had more severe seizures at the low doses of PTZ than did either control group. The 9 day old animals did not differ according to prenatal treatment.

These data are consistent with the data that show more seizures to audiogenic stimuli in alcohol exposed rats. They further argue that there are only transient increases in seizure thresholds for PTZ and are consistent with the report that showed decreased PTZ mortality in postweanling rats. The reasons for the recovery after initial increased susceptibility are not clear and should be the topic of further study.

NEUROTOXICITY III

360.1 INTRACISTERNAL METHOTREXATE SPECIFICALLY REDUCES CORTICAL METHIONINE SYNTHETASE ACTIVITY
P.A. Bradshaw, Z.-H. Zhang*, S.R. Snodgrass. Neurology Res., Childrens Hospital of Los Angeles,and USC School of Medicine, Los Angeles, CA 90054

In humans, repeated CNS exposure to methotrexate (MTX) during chemotherapy produces a white-matter lesion which we believe may result from impaired methylation reactions, although other mechanisms have been suggested. In several experiments we have found that rat brain methionine synthetase (Met S) activity is reproducibly reduced in the cortex and cerebellum, but not the hippocampus or striatum, after repeated intracisternal(IC) injections of MTX. This reduction is dose-dependent in response to MTX.

To determine the specificity of the effect of MTX on Met S, rats were given 200ug MTX IC on alternate days for two weeks (controls received vehicle). The activities of ATPase, choline acetyl transferase (CAT) and Met S were measured in each cortex and cerebellum. Cortical Met S activity decreased by 20% relative to controls (control (8) 2.98±0.38 nmol/hr/mg protein vs MTX (8) 2.39±0.30 nmol/hr/mg, $p<0.005$). However, Na,K ATPase (control 58.3±4.9 vs MTX 53.0±8.7 umol/hr/mg), Mg ATPase (control 6.7±0.8 vs MTX 6.4±0.5 umol/hr/mg) and CAT (control 165±37 vs MTX 169±34 pmol/min/mg) activities in the same tissues were unchanged. Met S activity in the cerebellum was reduced, but the difference was not statistically significant (control 3.12±0.48 vs MTX 2.76±0.49 nmol/hr/mg). Neither Na,K ATPase (control 44.8±4.8 vs MTX 44.7±6.6 umol/hr/mg) nor Mg ATPase (control 6.8±1.0 vs MTX 6.5±1.1 umol/hr/mg) were changed in the cerebellum by the MTX treatment. Thus it appears that Met S is specifically reduced while other enzymes are not affected.

Because Met S activity did change, we measured the levels of S-adenosyl methionine (SAM) in the same tissues. MTX treatment did not alter SAM concentration in either cortex (control 2.77±0.20 vs MTX 2.76±0.19 ng/g tissue) or cerebellum (control 4.08±0.37 vs MTX 4.28±0.26 ng/g).

The MTX effect on Met S is small but reproducible, and does not appear to explained by effects of MTX on DNA synthesis. Elucidation of the MTX white matter syndrome may be difficult in rats because the rodent brain contains relatively little white matter. Nitrous oxide, which inhibits Met S, will produce white matter lesions in primates, but fails to do so in rats.

360.2 BEHAVIORAL AND NEUROCHEMICAL DEVELOPMENT AFTER PRENATAL EXPOSURE TO METHYL PARATHION IN RATS. R.H. Rech, R.C. Gupta*, P. Welsch* and J.E. Thornburg. Dept. of Pharmacol./Toxicol., Michigan State Univ., East Lansing, MI 48824.

The purpose of this study was to determine the effects of the organophosphate methyl parathion (MPTH) on development of behavior and brain neurochemistry in rats exposed in utero. MPTH was administered p.o. to the dams in a dietary supplement of peanut butter (1.0 mg/kg) or gavaged in peanut oil (1.5 mg/kg) daily from day 6 through day 20 of gestation. Pups were cross-fostered at birth to untreated mothers.

A variety of behaviors examined in the offspring at various ages that are sensitive to developmental defects induced by other drugs were not significantly impaired by either dose of MPTH. These behaviors included neonatal reflexes, locomotor activity, rotarod or maze performance and avoidance behavior. Operant behavioral data using a mixed schedule suggests that subtle impairments of this behavior are produced by MPTH.

In parallel studies the effects of MPTH on the postnatal developmental patterns of acetylcholinesterase (AChE), choline acetyltransferase (ChAT), sodium-dependent, high-affinity choline uptake in synaptosomes (HACU) and muscarinic receptor binding in frontal cortex, striatum, and hippocampus were determined. Only the 1.5 mg/kg dose of MPTH altered the developmental pattern of AChE and ChAT in rat brain. In 7, 14, 21 and 28 day-old offspring AChE activity was reduced and ChAT activity increased in frontal cortex, hippocampus, striatum and brainstem of MPTH-exposed animals. The development of HACU by synaptosomes was not affected by MPTH. Thus, subchronic prenatal exposure to MPTH altered postnatal development of the cholinergic neurons in brain but had little effect on a variety of behaviors with the exception of an operant paradigm. (Supported by USPHS grant ES02256.)

360.3 LEAD INDUCED DYSFUNCTIONS OF CNS DOPAMINERGIC TRANSMISSION. S. Govoni, L. Lucchi*, R.A. Rius*, P.F. Spano and M. Trabucchi. Dept. of Pharmacology and Pharmacognosy, University of Milan, Italy.

Chronic lead intoxication induces several neurological abnormalities including restlessnes, irritability and other behavioural and psychological alterations. Recently, concern has arisen on the possible neurotoxic effects of low lead concentrations in the environment. In fact a correlation between behavioural disorders and chronic lead exposure has been proposed. In particular, blood and urine lead levels were found to be higher in hyperactive children in respect to control population. The hypothesis of a relationship between childhood hyperactivity and lead exposure, based on epidemiological studies, has been tested experimentally by exposing laboratory animals to lead according to various protocols but more frequently through the diet or the drinking water. In view of the motor hyperactivity induced by lead also in laboratory animals, a particular attention has been given in neurochemical studies to lead induced alterations of brain catecholamine metabolism. The aim of the present investigation was to study the effect of lead exposure on brain dopaminergic transmission. Pregnant Sprague Dawley rats were given a 2.5 g/L lead acetate solution in the drinking water. After birth, the offspring received the same drinking solution which had been supplied to their mothers. Brain catecholamine concentrations were measured by HPLC with electrochemical detection. Dopamine receptor characteristics were studied using ^{3}H-Spiroperidol, ^{3}H-Propylnorapomorphine or ^{3}H-Sulpiride as ligands. The results indicate that dopaminergic transmission is differentially affected by lead in functionally different brain dopaminergic structures. In particular, dopamine turnover and uptake are reduced in striatum where a subset of dopamine receptors shows adaptive changes. Opposite trends were observed in nucleus accumbens. The results are in favor of a dopaminergic dysfunction in rats exposed to lead concentrations of clinical relevance in humans.

360.4 METABOLIC ABNORMALITIES IN TRIETHYLTIN TREATED RATS. R.M. Rocco*, J.B. Blumberg and V.S. Sapirstein* (SPON: J.B. Blumberg). Dept. of Pharm. and Toxicology, Northeastern University, Boston, MA 02115 and Dept. Biochem., E.K. Shriver Center, Waltham, MA 02154.

Triethyltin (TET) is a potent neurotoxic agent which selectively interferes with myelinogenesis when administered postnatally. The mechanism of this action is unknown but may be related to the metabolic specialization of oligodendrocytes for myelin lipid synthesis. We tested the effects of TET on myelin-associated lipid synthesis in 20 day old rats which had received 3 consecutive ip injections of TET at 2 mg/kg on postnatal days 5, 6 and 7. TET treated rats had body and forebrain weights 85.5 and 49.6% of littermate controls. Total protein/g wet weight of forebrain was 116% of controls. Myelin yield/mg total protein was 65.2% of control.

Forebrain slices were incubated for 1 h in Krebs buffer with (U-^{14}C)glucose followed by isolation of myelin. Total lipids were extracted and separated by silicic acid chromatography. Incorporation of label into total myelin lipids was 902±81 (SEM) cpm/mg membrane protein for control compared to 614±70 for the TET ($p<0.05$). No changes were seen in the incorporation into myelin neutral lipids or cerebrosides, however, the phospholipid (PL) fraction in the controls contained 255±17 cpm compared to 172±17 for the TET. Incorporation of label/ug phosphorus into the glycerol moiety was shown by alkaline methanolysis to be reduced to 71.9% of control. When (^{3}H)acetate was used as a carbon source, a slight increase in incorporation was seen in the myelin PL fraction. Incorporation of glucose into myelin PL, unlike with acetate, occurs through conversion of glucose by glycolysis into dihydroxyacetone phosphate (DHAP). Conversion of DHAP into PL involves glycerol 3-phosphate dehydrogenase (G3PD) which converts DHAP into glycerol backbone of PL. G3PD is an enzyme known to be enriched in oligodendrocytes. We found the activity of this enzyme to be 74.6±4.0 nmole/min/mg protein in control compared to 43.9±6.0 in the TET. The k_m for DHAP and the V_{max} in the control were 0.15 mM and 83 nM/min respectively compared to the TET values of 0.18 and 45. Heat treatment of the samples at 50°C for up to 10 min gave identical decay curves indicating similar isozyme composition.

Because of the decreased V_{max} and the lowered incorporation into the glycerol moiety of the myelin PL fraction, we feel that TET causes a selective defect in the ability of the developing brain to synthesize membrane lipids at the level of DHAP. Consistent with this view is our previous report showing that transketolase, a rate limiting step in the synthesis of glyceraldehyde 3-phosphate which is then converted to DHAP, is also reduced in activity in TET treated animals.

Supported by BRSG Grant S07RR05830, NS16186 and HD05515.

360.5 INCREASED BEHAVIORAL REACTIVITY IN ADULT RATS FOLLOWING NEONATAL TRIETHYL LEAD ADMINISTRATION. R.M. Booze, C.F. Mactutus, Z. Annau and H.A. Tilson*, Lab. Behav. Neurol. Tox., NIEHS, Reseach Triangle Park, NC 27709 and The Johns Hopkins Univ. Baltimore, MD 21205.

Neonatal adminstration of triethyl lead (TEL) produces transient motor excitability followed by reduced activity and responsiveness through 21 days of age (Neurobeh. Tox. Teratol., in press). However, initial longitudinal behavioral evaluations found that neonatal TEL-exposure increased reactivity and decreased habituation in adulthood. The similarities between these alterations in reactivity and those found subsequent to septal-hippocampal lesions suggested the limbic system as a potential site of TEL neurotoxicity. Accordingly, the present study examined the long-term effects of neonatal TEL-exposure using tasks indicative of limbic system dysfunction.

The offspring of 16 Fischer-344 dams were neonatally injected with TEL chloride. Specifically, at 5 days of age one pup of each sex per litter was administered a single s.c. injection of either distilled water, 4.5- or 9.0-mg/kg TEL. The fourth pup of each sex was an undernourished control animal, which allowed comparison of non-specific effects of undernutrition on reactivity to those produced by TEL treatment. All animals were weighed weekly, but no testing occurred until adulthood. Two-way active avoidance testing began at 107 days of age. During an initial 120-sec apparatus habituation period, female 9.0-mg/kg TEL rats made a greater number of exploratory crossings. All animals then received 120 acquisition trials in either a contingent or non-contingent task. Under the contingency condition, no facilitatory effects of TEL on learning were found. However, both contingency conditions greatly enhanced intertrial interval responding, suggesting an increase in the reactivity of TEL-treated rats. Shock thresholds were assessed one week after two-way avoidance acquisition. Decreased responsiveness to shock was found in the 9.0-mg/kg TEL males, but only in those which had previously undergone the non-ontingent avoidance task. TEL also disrupted habituation to repeated testing in an originally novel hotplate testing environment (day 130), but no alterations in pain sensitivity were found. In each of these tasks, TEL-effects were detected in the absence of, or were greater than, effects due to early undernutrition. Preliminary neuropathological evaluations revealed hippocampal damage in TEL-treated rats; in particular, cell loss in area CA3 was found.

In summary, these findings suggest that 1) neonatal TEL-exposure produces long-term alterations in reactivity independent of undernourishment or sensory modality, and 2) persistent behavioral and neuropathological alterations follow neonatal TEL-exposure which are consistent with limbic dysfunction.

360.6 EFFECTS OF CHRONIC PYRUVIC ACID DERIVED TETRAHYDROISOQUINOLINES UPON RAT BRAIN STEM SEROTONIN. J.J. Hannigan*, C.L. Anderson* and W.P. Zeller*. (SPON:.M. Druse-Manteuffel). Dept. Pediatrics, Loyola Univ. Stritch Sch. of Med. Maywood, Il. 60153.

Subacute necrotizing encephalopathy (SNE; Leigh's disease) is an autosomal recessive disease of pyruvate dehydrogenase complex (PDHC) activity with progressive degeneration of the brain stem, cerebellum, striatum and characteristic sparing of the mammillary bodies. PDHC activity is necessary for entry of pyruvic acid into the Krebs cycle. Due to this impaired carbohydrate metabolism SNE patients have elevated pyruvic and lactic acid levels.

The progressive neurological degeneration has been largely attributed to decreased energy levels necessary for maintainance and repair of these brain regions. Serotonin (5-HT) acid metabolites have been reported to be absent in the later stages of SNE (Ebadi et al., J. Neurol. Neurosurg. Psychiatry. 32: 393-398, 1969).

The large amounts of pyruvic acid can condense with dopamine in the brain to form 1-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (1-CSAL). The primary O-methyl metabolite of 1-CSAL *in vivo* is 1-carboxy-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline (7-M-1-CSAL) (Origitano, T.C. Ph.D. Dissertation Loyola Univ. 1980).

The effects of these compounds upon brain stem 5-HT was determined in male Sprague-Dawley rats (33 days old) given either saline, 250 uM/kg/diem 1-CSAL or 250 uM/kg/diem 7-M-1-CSAL as a saline suspension for 4 days. One hour after the last dose the animals were dispatched by decapitation. The brain stem 5-HT was measured by reverse phase high performance liquid chromatography with electrochemical detection.

Brain stem 5-HT levels in the 1-CSAL treated animal were not significantly different from controls. 7-M-1-CSAL however resulted in significantly lower brain stem 5-HT when compared to both the control and 1-CSAL animals.

Group	Saline	1-CSAL	7-M-1-CSAL
n = 6			
5-HT			
ug/g ± s.e.m.	0.47±0.06	0.47±0.04	0.25±0.09*

*denotes $p < 0.05$ when compared to controls and 1-CSAL.

7-M-1-CSAL has been shown to be a potent depletor of rat brain stem 5-HT. It is speculated that 7-M-1-CSAL may be responsible in part for the neurotoxicity and decreased 5-HT content in SNE.

360.7 THE EFFECT ON THE AXONAL CYTOSKELETON OF ALUMINUM TARTRATE APPLIED LOCALLY TO THE SCIATIC NERVE. K. S. Kosik*, A. McCluskey*, D. J. Selkoe (Spon: C. Rasool). Harvard Medical School, Mailman Research Center, McLean Hosp., Belmont, MA 02178

Among the various neurotoxins which induce accumulation of intermediate filaments only aluminum results in a predominantly perikaryal accumulation of neurofilaments. However, involvement of the proximal axonal segment early in the course of experimental aluminum intoxication (Troncoso, J. C., et al, Ann. Neurol. 12:278, 1982) has suggested an analogy with the proximal axonal neurofilamentous swellings of ßß'-iminodipropio nitrile (IDPN) toxicity. Since local application of IDPN to the sciatic nerve has been shown to induce a local reorganization of axoplasmic elements (Griffin, J. W., et al, Neurosci. 3:557, 1983) we looked for the occurrence of a similar phenomenom in aluminum neurotoxicity. Previously we found that focal aluminum induced neurofibrillary lesions of the lumbar spinal cord of rabbits result in a depletion of choline acetyltransferase activity and an alteration in axonal transport of this enzyme.

A highly viscous solution of 1.5 M aluminum tartrate was prepared by raising the pH with sodium hydroxide to approximately 5.0. The sciatic nerves of 3 New Zealand white rabbits were surgically exposed, the perineurial sheath was incised and a 1 cm piece of Tygon tubing was placed around the nerve and filled with aluminum tartrate. After 48 hours, the rabbits were sacrificed and the sciatic nerves were immersion fixed in Karnovsky's fixative. Thin sections were prepared for electron microscopy by conventional techniques. Control nerve segments taken from sites proximal and distal to the aluminum application as well as from the contralateral sciatic nerve were similarly processed and examined. In the region of aluminum application cross sections revealed clusters of neurofilaments, most often seen at the periphery of the axon but occasionally forming irregular groups throughout the cross sectional area of the axoplasm. While some aluminum treated axons contained a central cluster of microtubules, central channels devoid of NF's (as seen with IDPN) were not detected. Central clusters of mitochondria also did not occur. Malorientation of some neurofilaments and microtubules away from the longitudinal axis of the axon was an additional feature in the aluminum-treated nerves. Our findings suggest that some axonal cytoskeletal reorganization occurs in aluminum toxicity but the mechanism of neurofilament accumulation may be distinct from that described for neurotoxins such as IDPN which produce predominantly axonal neurofilamentous swellings and a marked segregation of microtubules and neurofilaments. [Supported by NIH grants AG05268 (KSK) and K07NS00289 (DJS), and AG02126 (DJS)]

360.8 DOES PYRUVATE TREATMENT PREVENT ACRYLAMIDE NEUROTOXICITY? IMPLICATIONS FOR THE GLYCOLYTIC THEORY OF PATHOGENESIS. A.B. Sterman, D. Panasci* and W. Persons.* Department of Neurology, School of Medicine, SUNY at Stony Brook, Stony Brook, NY 11794.

Researchers have proposed that environmental neurotoxins act by inactivating glycolytic enzymes in the axon, producing energy deficits and axonal degeneration (Ann.Neurol. 5:501, 1979). To bypass this putative metabolic blockade, we treated acrylamide-intoxicated rats with high doses (3.5 gm/kg/d) of sodium pyruvate, the end product of glycolysis. We evaluated neurotoxicity using our quantitated neurobehavioral examination (Neurobehav. Toxicol. Teratol. 4:567, 1982) and quantitated morphology (J. Neuropathol. Exp. Neurol. 42:166, 1983). Four groups of 12 rats were used: control (C); pyruvate (P); acrylamide (A), and acrylamide plus pyruvate (A-P). The C and P groups did not differ. Compared to the A group, the A-P group showed a significant delay in onset of disease on 1 of 6 neurobehavioral subtests ($p < 0.05$; Wilcoxon test) and trends on others. Measures of the rates of disease progression did not differ on any of 8 subtests. Light microscopic morphometric study of scored abnormalities in DRG cell bodies showed the A and A-P groups displayed equivalent abnormalities: granular inclusions (mitochondria), Nissl banding, eccentric nucleus, and chromatolysis-like triad. In both groups only a small number of fibers in the tibial nerve branches and distal tibial nerve showed degeneration. While showing only a minimal protective effect, our data do not refute the glycolytic hypothesis; rather, they imply that inactivation of glycolytic enzymes alone is not a sufficient explanation and suggest the need to consider the entire neuron, both axon and cell body, to understand pathogenesis. (Supported by ES-02650 and the Veterans Administration)

360.9 SUBTLE NEUROTOXICITY OF PRENATAL EXPOSURE OF RATS TO A 1:1 MIXTURE OF 2,4-DICHLOROPHENOXYACETATE (2,4-D) and 2,4,5-TRICHLOROPHENOXYACETATE (2,4,5-T) ON POSTNATAL BEHAVIORAL AND NEUROCHEMICAL DEVELOPMENT. F.K. Mohammad* and V.E.V. St. Omer. Lab. of Neurobehavioral toxicology and neuropharmacology. Univ. of Missouri, College of Veterinary Medicine, Columbia, MO 65211.

"Safe" levels of prenatal exposure of animals to Agent Orange or other phenoxy herbicides are currently documented on the basis of offspring survival, physical growth, maturation, and anatomical defects. No consideration is given to possible subtle neurologic consequences. This study describes the subtle neurobehavioral and neurochemical alterations of the overtly normal developing rat following prenatal exposure to phenoxy herbicides.

Pregnant Sprague-Dawley rats were orally administered 50(Group I, 100(Group II) or 125(Group III) mg/kg of a 1:1 mixture of 2,4-D and 2,4,5-T or 2 ml/kg of peanut oil vehicle (control group IV) on gestational days 6 through 15. Rats delivered normally. Offspring were culled to 4 males and 4 females on postnatal day (PND) 1, and weaned on PND21. Group II and III mothers gained significantly ($P<0.05$) less weight during pregnancy and delivered fewer offspring than Groups I and IV. Gestational length was unaffected by 2,4-D:2,4,5-T treatment. Offspring were examined for body weight differences and physical maturation (incisor eruption, eye opening, testes descent and vaginal opening). Treatment had no effect on growth and maturation. No gross anomalies were visible. Examination of ontogeny of behavior (surface righting reflex on PND2-5, negative geotaxis on PND7-11 and 15-17, olfactory discrimination of home cage odor on PND9-11 and swimming on PND7-15 & 21) indicated treatment effects. Surface righting reflex, negative geotaxis (PND15-17) and swimming ability were significantly delayed in all treated Groups. Olfactory discrimination, and negative geotaxis (PND7-11) were significantly delayed in Groups II and III. Neurochemical measures of whole brain norepinephrine (NE) and dopamine (DA) turnover rates on PND3,9,15 in Groups I, II and IV and cerebrum levels of glutamate and GABA on PND1 in Groups I, III and IV indicated delayed ontogeny of some neurotransmitters. Brain DA, but not NE turnover rates (ng/gm/hr) of 3 day old rats in Group I(19.3±3.0) and Group II(22.7±3.6) were significantly lower than those of controls (31.5±3.0). No alterations in catecholamine turnover rates occurred on PND9 and 15. Postnatal day 1 cerebrum glutamate levels in Group I (3.8±0.1) and Group III(3.6±0.2) were significantly lower than control value (4.2±0.1). GABA levels were unaffected by treatment. The data indicated that prenatal exposure to a "maternally safe and non-teratogenic" dose (Group I) of 2,4-D:2,4,5-T produced in the overtly normal developing rat adverse neurologic effects.

Support: Veterinary Medical Research Council, Univ. of MO.

360.10 BEHAVIOURAL AND NEUROCHEMICAL CHANGES IN OFFSPRING OF RATS EXPOSED TO METHYL MERCURY DURING GESTATION. V.Cuomo[+1], L.Ambrosi[+2], R.Cagiano[+1], N.Brunello[3], and G.Racagni[3]. [+1]Institute of Pharmacology, University of Bari, [2]Institute of Occupational Health, Bari and [3]Institute of Pharmacology and Pharmacognosy, University of Milan, Italy.

Neonatal activity testing revealed abnormal patterns of locomotor development in methyl mercury chloride (MMC) exposed pups. Since neurochemical correlates are lacking, in the present study we have investigated both the behavioural and biochemical effects of prenatal administration with MMC. On day 8 of gestation, pregnant Sprague-Dawley rats were given 8 mg/kg of MMC dissolved in distilld water. MMC was administered by means of intragastric intubation. The percentage of animals giving birth was the same for treated and control groups. Lenght of gestation, litter size and body weight of MMC-exposed offspring were unaffected by MMC treatment. No significant differences in locomotor activity levels between MMC treated pups and controls on either postnatal day 15 or 22 were found. However, at 15 and 22 days of age the stereotyped behaviour as well as the effects on locomotor activity elicited by a challenge dose of apomorphine (0.5 - I mg/kg s.c.), a dopamine receptor stimulating agent, were significantly potentiated in MMC-pretreated pups. Neurochemical experiments showed that at 15 days of age there was a trend to an increase in the number of ^{3}H-spiroperidol binding sites in striatal memebranes of MMC-pretreated rats which reached statistical significance at the 22nd day after birth. On the other hand, prenatal exposure to MMC did not influence the effects of a challenge dose of clonidine (0.025 mg/kg s.c), an alpha-2 adrenoreceptor agonist, on locomotor activity of 15 and 22 days old rats. Neurochemical experiments showed that at 15 and 22 day of age, neither the affinity nor the density of alpha-2 adrenoreceptors in cortex were affected by the prenatal administration of MMC. Therefore, the present data indicate that prenatal exposure to MMC in rats produces alterations in the behavioural response of offspring to a dopaminergic agent (apomorphine) whereas it does not influence the behavioural responsiveness to a noradrenergic agent (clonidine). These results are in line with neurochemical data. Particularly, the behavioural alterations in the response to apomorphine presumably result from the production of dopamine receptor supersensitivity induced by MMC.

360.11 A BIOCHEMICAL MEASURE OF NEUROTOXICITY OF METALS IN REGIONS OF THE BRAIN OF ADULT RATS USING ALTERATION IN LYSOSOMAL ENZYME ACTIVITY AND LIPID PEROXIDATION RATES. B. Callahan*, M. Cleaves*, R. Schatz* and D.R. Brown*. (SPON: J. Neumeyer). Toxicol. Prg., College Pharm. & Allied Health, Northeastern Univ., Boston, MA 02115.

Behavioral toxicity and effects in the whole brain have been described for a variety of neurotoxic agents but few general approaches are available to identify injury to specific areas in the brain. In this dose response study, 2 biochemical markers of cell injury, lipid peroxidation and lysosomal enzyme activity (ß-galactosidase activity), were used to investigate relative responses of 6 brain regions to a toxic metal. Adult rats were dosed with 4 or 8 mg/kg thallous acetate i.p. Behavior was measured on day 7 and animals sacrificed on day 8.

The data show that:

1) Thallium exposure increased the rate of lipid peroxidation in selective regions of the brain, i.e. cerebellum, brain stem, cortex and striatum with high doses of thallium (8 mg/kg). The lower dose (4 mg/kg) affected only the cerebellum and brain stem.

2) Thallium exposure increased the activity of ß-galactoside in all brain regions tested except striatum at high dose. At the lower dose only cerebellum and brain stem activities were altered.

3) Thallium altered the clustering tendency of certain mode of rat behavior without affecting other behaviors.

4) The regional biochemical alterations were dose related. Changes in specific behavior modes were also dose related.

These findings suggest that comparison of biochemical changes with behavioral changes will permit identification of heavy metals, as well as other toxic agents, which may produce neurological damage at low levels in the rat.

360.12 TRIMETHYLTIN ADMINISTRATION ALTERS CORTICAL AND HIPPOCAMPAL EEG POWER SPECTRA DURING SLOW-WAVE AND RAPID EYE MOVEMENT SLEEP. Kathleen R. Stratton*, Gerald A. Young and Christine U. Eccles. Dept. of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.

The hippocampus exhibits characteristic patterns of electrical activity seen in the EEG during slow-wave sleep (SWS) and rapid eye movement (REM) sleep. Pharmacological agents such as opioids disrupt the sleep cycle. Thus, EEG measurements taken during various stages of the sleep cycle could prove to be sensitive indicators of neurotoxicity.

Oral administration of trimethyltin (TMT) produces neuronal loss which is most prominent in the hippocampal formation. We attempted to determine whether TMT intoxication disrupts the pattern of electrical activity as delineated by power spectral densities in both the hippocampus and cortex. Male Sprague Dawley rats were implanted with stainless steel bipolar electrodes in the dorsal hippocampus and with cortical screw electrodes attached to the contralateral skull. After a two week recovery period the animals were habituated to the recording cage for at least three days. The recording cage served as the home cage for the duration of the experiment. EEG episodes of SWS and REM sleep were recorded, stored on FM tape, digitized at a rate of 100 per second, and analyzed on a Nicolet Med-80 computer. Spectral analysis was done on 50 sec samples of EEG for each of the sleep states. After control recordings, 7.5 mg/kg TMT was administered by oral intubation.

As early as one day after TMT, the peak frequency of power density during REM sleep was decreased from control recordings. This effect persisted for at least 21 days. Some animals showed a loss of the generation of rhythmic slow activity (RSA) typically seen during REM sleep. These same rats previously displayed hippocampal RSA before TMT treatment. Alterations in the power distribution of the SWS spectra also appeared and began to develop after the alterations in the REM sleep spectra were already manifest. SWS spectra of TMT-treated rats revealed a much greater concentration of the total power in lower frequencies than that seen in control recordings. These alterations in the sleep spectra were seen prior to and persisted longer than the overt behavioral manifestations of TMT intoxication. Thus, disruption of the hippocampal and cortical EEG during SWS and REM sleep as detected by power spectra analysis appears to be a sensitive indicator of TMT intoxication.

360.13 EARLY COGNITIVE DEFICIT IN PHENYLACETATE-INDUCED MATERNAL PHENYLKETONURIA IN RATS. A. Rabe, Y.H. Loo*, P. Wang*, R. Fersko* and A. Potempska*. New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314.

Phenylacetate (PA), a major metabolite of phenylalanine, may be the primary cause of brain dysfunction in phenylketonuria (PKU), as proposed by Loo et al. in 1978 (In Myelination and Demyelination, edited by J. Palo, Plenum Press, 453-469). We have now extended this hypothesis to maternal PKU. We simulated maternal PKU in the rat with two different dosage schedules: (1) combined pre- and early postnatal exposure to PA during a period that corresponds to fetal brain development in man, and (2) solely prenatal exposure. While both treatment schedules produced a learning deficit, the effects of the longer exposure were more pervasive. Neither schedule of treatment produced changes in activity or exploration.

Pregnant Sprague-Dawley rats from gestation day 9 to 20 received subcutaneously (s.c.) a continuous infusion of 6 µmol/g/20 ml/24 hr., which produced a steady plasma level of 0.25 - 0.55 µmol/ml of unconjugated PA. On postnatal (PN) day 3, each litter was culled to 8 pups and transferred to a normal surrogate mother. The pups assigned to the pre- and postnatal schedule (n= 10 litters) received additional PA from PN 3 - 8 in two daily s.c. injections of 1.5 µmol/g, increased by 0.5 µmol/g on every third day. The pups assigned to the prenatal group (n= 5 litters) received no postnatal PA injections. The control litters (n= 11 for the pre- and postnatal treatment; n= 10 for prenatal) were exposed to similar treatment schedules with physiological saline.

Starting on PN 17, half of the pups from each litter were given acquisition and then reversal training on a left-right discrimination in a T-maze. On PN 30, the other half of the pups were tested for ambulation and head dipping in a hole-board. The effects of PA on these three measures were evaluated by comparing the litters exposed to each of the two PA schedules with those exposed to the corresponding saline treatments.

The litters exposed to PA pre- and postnatally showed an acquisition ($p<.025$), as well as a reversal ($p<.01$) deficit (i.e., made more errors than controls). The litters exposed to PA only prenatally had a reversal deficit ($p<.025$) without an acquisition deficit. The magnitude of the reversal deficit did not differ for the two PA treatment schedules. Neither PA treatment schedule produced significant changes in either the amount of ambulation or in the number of head dips.

(Supported in part by NIH grants 1 R01 HD 16153 and 06843.)

360.14 L-DOPA PREVENTS THE EFFECTS OF DSP 4 ON DEVELOPING AND MATURE NORADRENALINE NEURONS. G. Jaim-Etcheverry and L. M. Zieher*. Instituto de Biología Celular, Facultad de Medicina, 1121 Buenos Aires, Argentina.

The compound N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP 4) markedly impairs noradrenaline (NA) uptake by central and peripheral NA neurons and depletes the endogenous stores of the transmitter. These effects are reversible at the periphery but seem to be permanent in the brain. Moreover, DSP 4 injected during ontogenesis produces long-term changes in the development of central NA neurons, i.e., a denervation of the cerebral cortex and the spinal cord and a hyperinnervation particularly evident in the cerebellum[1].

To determine the influence of intraneuronal monoamine levels on the actions of DSP 4, L-DOPA, the precursor amino acid in catecholamine synthesis, was given before the neurotoxic compound. Newborn rats were injected immediately after birth with DSP 4 (50 µg/g sc) alone or 20 min after L-DOPA (100 µg/g sc). In rats killed at 45 days of age, the changes characteristically observed after DSP 4 were markedly counteracted by L-DOPA. NA depletion from the cerebral cortex and the spinal cord was less important and NA did not increase markedly in the cerebellum as it did after DSP 4 alone.

In adult mice, L-DOPA (100 mg/kg sc) 60 min before DSP 4 (50 mg/kg ip), completely prevented the depletion of heart NA caused by DSP 4 when given alone. The protection afforded by L-DOPA was lost when the activity of peripheral DOPA-decarboxylase was inhibited by carbidopa (170 mg/kg ip) injected before L-DOPA and DSP 4. When DSP 4 was given after L-DOPA, the reduction of NA levels in the cerebral cortex was less important than after DSP 4 alone.

These results indicate that the monoamine precursor L-DOPA, most probably after being decarboxylated to catecholamines, prevents to a great extent the changes that the neurotoxic compound DSP 4 produces in developing and mature NA neurons of rodents.

[1]Jaim-Etcheverry,G. and Zieher, L.M., Brain Res., 188:513,1980.
(Supported by grants from CONICET and SUBCYT, Argentina)

360.PO DIFFERENT PATTERNS OF HIPPOCAMPAL LESION INDUCTION IN RATS AS A RESULT OF TRIMETHYLTIN EXPOSURE AT DIFFERENT POSTNATAL AGES. L.W. Chang, D.A. Brown* and R.S. Dyer. Dept. of Pathology, Univ. of Ark. for Medical Sciences, Little Rock, AR 72205 and Division of Neurotoxicology, EPA Health Effects Laboratory, Res. Triangle Park, NC 27711.

While the pathological effects of trimethyltin (TMT) compounds on the limbic system are well recognized, the toxic impact of TMT on the developing nervous system is still relatively unknown. Our present investigation was designed to study the pathologic impact of TMT on the developing hippocampus as a result of exposure at various ages of postnatal life. Sprague-Dawley rats were mated and allowed to deliver at term. The days of birth for the pups were recorded and were designated as postnatal day 1 (PND 1). Pups were injected (i.p.) on PND 1, 3, 6, 7, 8, 9, 10, 11, 13, 15, 20, 25 or 30 with a single administration of TMT at a dose of 6.0 mg/kg b.w.; control pups were injected with equal volumes of saline solution. Animals injected between PND 1 and 11 were sacrificed at PND 15, 21 and 31; animals injected between PND 13 and 15 were sacrificed at PND 21 and 31. Animals injected after PND 20 were sacrificed 1-2 weeks after injection to allow appropriate time for lesion development. While there was no significant pathological damage in the hippocampal formation of rats injected on PND 1 and 3, increased neuronal necrosis was observed in the Ammon's horn pyramidal neurons in animals injected between PND 6-15. The progression of neuronal involvement was $CA_{3b} \rightarrow CA_{3a,b} \rightarrow CA_{3(a,b,c)} \rightarrow CA_3$ and $CA_2 \rightarrow$ entire Ammon's horn ($CA_{1,2,3}$). This pattern of pathological lesion was in good concert with morphological and functional development of the hippocampal formation in various stages of postnatal life (Stirling, R.V. and Bliss, T.V.P., Prog. Brain Res., 48:191-198, 1978). A gradual reduction of toxic sensitivity was observed after PND 20, and the pathological pattern assumed that of adult animals as reported in previous studies. Based on our present study, it was apparent that the hippocampus of rats were relatively insensitive to TMT toxicity in early postnatal age. However, the hippocampal formation, particularly the Ammon's horn, became increasingly vulnerable to TMT as the animals matured. The most sensitive postnatal period appeared to be PND 11-15 leading to total destruction of the Ammon's horn by TMT. Upon full maturation of the nervous system (after PND 20), the sensitivity to TMT toxicity again became greatly reduced. Our present findings suggest that the maturational and functional stages of the nervous system may play an important role in the induction of pathological lesions in the hippocampal formation as a result of TMT intoxication. (Supported by EPA CR809360-01. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

360.PO TULLIDORA (BUCKTHORN) NEUROPATHY: AXONAL DIAMETER IS RELATED TO DEMYELINATION. A. Hernández-Cruz* and E.J. Muñoz-Martínez. Depto. de Fisiología y Biofísica. Centro de Investigación y de Estudios Avanzados. Apto. Postal 14-740. México, D.F. 07000. MEXICO.

Human patients accidentally poisoned with tullidora *Karwinskia humboldtiana* fruits develop flaccid paralysis but the somatic sensation appears to be preserved. Accordingly, cat muscle nerves are more affected than cutaneous ones although it is not known whether muscle sensory fibers are relatively spared compared to motor axons. On the other hand, thicker fibers are more intensively demyelinated in the tullidora (buckthorn) neuropathy than thinner ones. Thus, the goals of this work were 1) to find out whether muscle afferents and motor axons of the same nerve are differentially affected by tullidora and 2) to establish whether the preference of tullidora toxins for the motor system might reflect that, on the average, alpha-motor axons are thicker than other fibers. The fiber diameter in normal cats and the severity of demyelination in treated cats were estimated from the conduction velocity of single fibers.

The conduction velocity (CV) of medial gastrocnemious (MG) and Soleus (Sol) motor axons, MG afferents and sural fibers was measured in 5 normal cats and 6 cats showing hind limb paralysis 4-6 weeks after a single oral dose of tullidora extracts. The experiments were performed under barbiturate anaesthesia stimulating the nerves and recording single unitary potentials from spinal root filaments. The sciatic nerve was taken out at the end of the experiments to measure the conduction distance.

In normal cats, MG motor axons had the highest average CV ($\overline{CV}$) whereas the sural had the lowest; the $\overline{CV}$ values of Sol motor axons and MG afferents were in between. In general, $\overline{CV}$ values were positively correlated with the proportion of fast (>80 m/sec) fibers in each nerve. In treated cats, $\overline{CV}$ was decremented in all studied nerves but the decrement was larger in faster fibers so that a strong positive correlation ($r=0.98$) was found between normal CV values and $\overline{CV}$ decrements in treated cats.

We conclude that the magnitude of demyelination in buckthorn neuropathy is related to the fiber diameter regardless of the fiber functional type.

360.PO TRIMETHYLTIN INDUCED CHANGES IN THE NEONATAL HIPPOCAMPUS - A HISTOPATHOLOGICAL AND ULTRASTRUCTURAL STUDY. N. Newton*, L.W. Chang, A. Molinaro*, and D.A. Brown* (SPON: T. Schoultz). Dept. of Pathology, Univ. of Ark. for Med. Sciences, Little Rock, AR 72205.

Trimethyltin (TMT) as a potent neurotoxicant, especially on the limbic system, is well recognized. Our present study is to provide information on the pathological impact of TMT on the neonatal nervous system. Neonatal rats (Sprague-Dawley) were injected (i.p.) with trimethyltin chloride at a dose of 7.0 mg/kg b.w. on postnatal day 11 (PND 11). Control animals were similarly injected with equal volumes of saline solution. Animals, in groups of 5, were sacrificed on PND 15 and PND 24. At sacrifice, animals were perfused intracardially with saline solution followed by 2.5% buffered glutaraldehyde. Brains were carefully removed. Left hemispheres of the brains were further fixed in 10% buffered formalin and prepared for light microscopy examination. The hippocampus from right hemisphere of the brains were dissected out and processed for electron microscopy. Extensive destruction of the hippocampal formation was observed 4 days after TMT exposure (PND 15). The Ammon's horn was much more affected than the fascia dentata. Most of the pyramidal neurons appeared to be necrotic displaying pyknotic nuclei and eosinophilic cytoplasm. Many of the surviving neurons acquired a swollen appearance with eccentric nuclei and distended cytoplasm. Necrosis of the granule cells was also present in the fascia dentata. However, significant numbers of the granule cells were still morphologically intact. Electron microscopy examination revealed edematous changes in the granule cells. Accumulation of lysosomes and degenerated organelles were found in the surviving granule cells and pyramidal neurons. By PND 24, most of the necrotic activity in the hippocampal formation had subsided. At light microscopic level, the fascia dentata returned to a normal appearance while the entire Ammon's horn had disappeared. Significant thinning of the cerebral cortex, atrophy of the hippocampus, and distended ventricles were observed. By means of electron microscopy, lysosomal accumulation was still observed in many granule cells of fascia dentata. Multifoci of electron-dense materials were observed in the fascia dentata and in the Ammon's horn. Close examination revealed that these deposits had a filamentous, crystalloid appearance. They are believed to be the result of dystrophic calcification changes of the necrotic tissues. Active removal of these calcified necrotic materials by macrophages and proliferation of astrocytes and their fibers were also observed. Our study revealed that neonatal nervous system was much more sensitive to TMT toxicity than the adult nervous system leading to extensive destruction of the Ammon's horn in a relatively short period of time. While there was some initial injury to the fascia dentata, the pathological impact on the granule cells was much less than that on the Ammon's horn. (Supported by EPA CR809360-01.)

360.PO ENERGY METABOLISM IN AN EXPERIMENTAL NEUROPATHY INDUCED IN RATS BY BROMOPHENYLACETYLUREA. S. Brimijoin and K.P. Mintz*. Dept. of Pharmacology, Mayo Clinic, Rochester, MN 55905.

In rats, p-bromophenylacetylurea (BPAU) induces severe, long-lasting damage to the distal parts of peripheral nerves. A single dose (200 mg/kg, i.p.) is followed by 5-7 days of normal neurologic function, then by hindlimb paralysis. We have previously correlated the neurotoxicity of BPAU with impaired switching from rapid anterograde to rapid retrograde axonal transport in the distal axon (Jakobsen and Brimijoin, 1981). We have now examined the possibility that this impairment is due to defective glycolysis, as suggested by recent reports that neurotoxic hydrocarbons inhibit glyceraldehyde-3-phosphate dehydrogenase (GADH) and phosphofructokinase (PFK). We first tested crystalline preparations of these glycolytic enzymes (Sigma Chemical) but found no inhibition by saturated aqueous solutions of BPAU. Because the active toxin could be an unknown metabolite, we next measured GADH and PFK activities in nerves from variously treated Sprague-Dawley rats. Pieces of sciatic nerve were taken from proximal and distal regions and were homogenized in cold Na-phosphate (50 mM, pH 7.4) containing 1 mM dithiothreitol. Homogenates were centrifuged at 10,000 x g for 10 min at 4° and were then immediately assayed spectrophotometrically. The results (Table 1) showed essentially identical enzyme activity in control rats, rats treated with vehicle, and paralyzed rats treated with BPAU two weeks earlier. Evidently the neurotoxicity was not due to inhibition of GADH or PFK. Recognizing that BPAU could have impaired energy metabolism in other ways, we examined ATP and creatine phosphate (CP). For this purpose, nerves from anesthetized rats were rapidly chilled and homogenized in ice-cold 0.4 N $HClO_4$. ATP and CP, assayed by the firefly-luciferase method, were found in the same amounts in all three groups (Table 1). We conclude that BPAU neuropathy does not begin with an attack on the means of generating high energy phosphates in peripheral nerve. (Supported by NIH grant NS 18170.)

Table 1. Effect of BPAU on Activities of Glycolytic Enzymes and Content of High Energy Phosphates in Rat Sciatic Nerve.

	GADH[a]		PFK[a]		ATP[b]	CP[b]
	proximal	distal	proximal	distal		
Control	340	300	77	62	3.8	2.2
Vehicle	350	320	90	68	4.2	2.0
BPAU	360	330	108	75	3.8	2.1

[a] milliunits/mg protein; 5 rats/group; standard errors < 10%.
[b] μg/mg protein; 3 rats/group; standard errors < 20%.